US Army Corps
of Engineers
Construction Engineering
Research Laboratories

USACERL Technical Report 97/140
September 1997

Methods for Field Studies of Effects of Military Smokes, Obscurants, and Riot-Control Agents on Threatened and Endangered Species

Volume 2: Methods for Assessing Ecological Risks

by
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Smokes, obscurants, and riot-control agents constitute a diverse group of chemical compounds that are released into the environment during military training exercises. Concern has been expressed over the use of these compounds and how they may adversely affect threatened and endangered (T&E) species that reside on military reservations. To evaluate if smokes and obscurants present a hazard to T&E species, the appropriate data must be collected. These data must be adequate to evaluate both direct ecological effects (effects to T&E species that result from direct exposure to smokes or smoke residues) and indirect ecological effects (effects on T&E species that result from effects of smokes on habitats of T&E species or on species upon which T&E species depend). This report presents an approach for the selection of methods suitable to evaluate the ecological risks that smokes and obscurants present to T&E species, summarizes available sampling, survey, and toxicity testing methods, and outlines an approach for estimating risks based on weighing multiple lines of evidence.

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REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave Blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1997	Final

4. TITLE AND SUBTITLE
Methods for Field Studies of Effects of Military Smokes, Obscurants, and Riot-Control Agents on Threatened and Endangered Species, Volume 2: Methods for Assessing Ecological Risks

5. FUNDING NUMBERS
E74
4A162720
A896
TC6

6. AUTHOR(S)
B.E. Sample, T.L. Ashwood, B.A. Carrico, L.A. Kszos, M.S. Nazerias, T.L. Phipps, W.K. Roy, M.G. Ryon, E.M. Schilling, M. Smith, A.J. Stewart, G.W. Suter II, L.F. Wicker, and K.A. Reinbold

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
U.S. Army Construction Engineering Research Laboratories (USACERL)
P.O. Box 9005
Champaign, IL 61826-9005

8. PERFORMING ORGANIZATION REPORT NUMBER
TR 97/140, Vol. 2

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Strategic Environmental Research and Development Program (SERDP)
901 N. Stuart Street, Suite 303
Arlington, VA 22203

Directorate of Environmental Programs
ATTN: DAIM-ED-N
7701 Telegraph Road, Casey Bldg.
Alexandria, VA 22310-3862

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
Copies are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT (Maximum 200 words)

Smokes, obscurants, and riot-control agents constitute a diverse group of chemical compounds that are released into the environment during military training exercises. Concern has been expressed over the use of these compounds and how they may adversely affect threatened and endangered (T&E) species that reside on military reservations. To evaluate if smokes and obscurants present a hazard to T&E species, the appropriate data must be collected. These data must be adequate to evaluate both direct ecological effects (effects to T&E species that result from direct exposure to smokes or smoke residues) and indirect ecological effects (effects on T&E species that result from effects of smokes on habitats of T&E species or on species upon which T&E species depend). This report presents an approach for the selection of methods suitable to evaluate the ecological risks that smokes and obscurants present to T&E species, summarizes available sampling, survey, and toxicity testing methods, and outlines an approach for estimating risks based on weighing multiple lines of evidence.

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14. SUBJECT TERMS
threatened species
endangered species
smoke and obscurants
ecological surveys
military training

15. NUMBER OF PAGES
236

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

Foreword

The funding for this work was provided by the Strategic Environmental Research and Development Program (SERDP) under Work Unit E74, "Impacts of Military Chemicals on Endangered and Threatened Species." Congress established SERDP through Public Law 101-510 on November 5, 1990 (10 U.S.C. 2901-2904). SERDP is a joint multi-agency (Department of Defense, Department of Energy, and Environmental Protection Agency) effort to support environmental quality research, development, demonstration, and applications programs. Dr. Femi Ayorinde is the technical monitor, and Bradley P. Smith is Executive Director, SERDP.

Additional funding was provided by the Office of the Directorate of Environmental Programs (DAIM), Assistant Chief of Staff (Installation Management) (ACS(IM)) under 40162720A896, "Base Facility Environmental Quality," Work Unit A896-LL-TC6, "Threshold Impacts for Smokes and Obscurants on TES." The technical monitor was Phil Pierce (DAIM-ED-N).

The work was performed by the Natural Resource Assessment and Management Division (LL-N) of the Land Management Laboratory (LL), U.S. Army Construction Engineering Research Laboratories (USACERL). The USACERL principal investigator was Dr. Keturah A. Reinbold. Dr. William D. Severinghaus is Laboratory Operations Chief, CECER-LL. The USACERL technical editor was Linda L. Wheatley, Technical Information Team.

Authors other than Dr. Reinbold are members of the staff of the Environmental Sciences Division of Oak Ridge National Laboratory (ORNL) and performed work on this report under contract with USACERL. The authors are grateful for the technical reviews provided by Ruth Hull, Warren Webb, Elizabeth Will, and George Southworth of ORNL and Dr. David J. Tazik of USACERL. Manroop Chawla (USACERL) assisted with coordination of the report preparation and format, and Lisa Baron (ORNL) assisted with quality control.

COL James A. Walter is Commander of USACERL, and Dr. Michael J. O'Connor is Director.

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1 Introduction

Background

Smokes and obscurants and riot-control agents constitute a diverse group of chemical compounds that are released into the environment during military training exercises. There is concern that the use of these compounds may have adverse effects on threatened and endangered (T&E) species that reside on military reservations. To evaluate if smokes and obscurants or riot-control agents present a hazard to T&E species, the appropriate data must be collected. These data must be adequate to evaluate both direct ecological effects (effects to T&E species that result from direct exposure to smokes or smoke residues) and indirect ecological effects (effects on T&E species that result from effects of smokes and obscurants on habitats of T&E species or on species on which T&E species depend).

Objectives

The objectives of this report are to:

1. identify the types of data that are needed to evaluate risks that smokes and obscurants present to T&E species
2. present an approach for the selection of appropriate sampling methods
3. summarize available and appropriate methods
4. outline how data generated by these methods should be used to evaluate if smokes and obscurants actually present a risk to T&E species.

Approach

T&E species that may occur on military reservations include birds, mammals, reptiles, amphibians, aquatic and terrestrial invertebrates, fish, and plants. Data collection methods discussed in this report were derived from the literature and represent a range of those methods that may be used for specific groups of species or habitats. When preparing to collect data to perform a risk assessment for a particular T&E species, the actual methods used will depend on the life history

characteristics of that species and the characteristics of the area and habitats in which risks are being assessed. To ensure the appropriate data for risk assessment are collected and the most appropriate methods for the T&E species of interest are used, both persons knowledgeable about the T&E species of interest and those with experience in ecological risk assessment should be involved with or consulted during the development and implementation of any ecological risk assessment sampling plan for T&E species.

Mode of Technology Transfer

Information derived from this study will be reported to the Strategic Environmental Research and Development Program (SERDP) advisory group. This information will be incorporated into development of a risk assessment framework for natural resources on military lands. This report will be distributed to major military commands and to installations where smokes and obscurants (or riot-control agents) are used and endangered, threatened, or candidate species are known to occur or may be present. This information will assist assessment of potential impacts of smokes, obscurants, and riot-control agents on T&E species at military installations.

2 Ecological Risk Assessment

Ecological risk assessment (ERA) is a process that evaluates the likelihood and magnitude of adverse ecological effects that may occur or are occurring as a result of exposure to one or more stressors (EPA 1992a). For there to be an ecological risk, four elements must be present: a source of contamination (e.g., smokes and obscurants), a migration pathway for the contaminant to get from the source to the receptor (e.g., air, water, soil, and food), a receptor (e.g., T&E plants and animals), and an exposure route (e.g., ingestion, inhalation, and dermal contact). If any one of these components is not present, there is no ecological risk. Both chemical contaminants and nonchemical stressors such as habitat modification are evaluated in an ERA.

All ecological risk assessments should follow the standard paradigm outlined in the *Framework for Ecological Risk Assessment* (EPA 1992a). Under this paradigm, an ERA consists of four components: problem formulation, exposure assessment, effects assessment, and risk characterization.

In the problem formulation phase of an ERA, the goals, breadth, and focus of the assessment are established. To do this, the abiotic and biotic segments of the contaminated environment are described, the spatial extent of the problem is defined, chemicals responsible for the contamination are identified, endpoints appropriate to evaluate ecological effects of contamination are selected, and conceptual models are developed that describe pathways by which the contaminants move through the abiotic and biotic environment, expose plants and animals, and induce effects.

In the exposure assessment, the transport and transformation of contaminants and their contact with endpoint species are evaluated. Pathways by which plants and animals are exposed to contaminants are identified and quantified.

The purpose of the effects assessment is to qualitatively and quantitatively assess the relationship between contaminant exposure and effects on plants, animals, and ecosystems. Potential effects may be either direct or indirect. Effects of contaminants on plants and animals are evaluated using a combination of biological survey data, conventional toxicity data, and ambient media toxicity test data.

The final step in performing an ERA is risk characterization. In this phase, data from the exposure and effects assessments are combined to characterize the risks to assessment endpoints. In addition, assumptions, results, strengths, and weaknesses of analyses and associated uncertainties are summarized and explained. Risk characterization combines information concerning exposure to contaminants with information concerning effects of contaminants to estimate risks. Risk characterization for ERAs is performed by weight of evidence (EPA 1992a). That is, rather than simply modeling risks, ecological risk assessors examine all available data from chemical analyses, toxicity tests, biological surveys, and bioindicators to estimate the likelihood that significant effects are occurring or will occur and to describe the nature, magnitude, and extent of effects on the designated assessment endpoints. Chapter 9 describes an approach for estimating risks based on individual lines of evidence combined through a process of weighing.

A tiered or phased approach for ERA is recommended as suggested by other authors (Suter 1993; Wentsel et al. 1994). Wentsel et al. discuss a three-tiered approach that provides procedural guidelines for ERAs at contaminated Army sites targeted for cleanup. These phases would be relevant for other ERAs as well.

The purpose of a tiered approach is so the necessary work may be done to characterize the risk to an ecological system with an acceptable degree of uncertainty. Each tier includes phases for problem formulation, analysis, and risk characterization. Data collected in the analysis phase of each tier are evaluated, and a decision is made concerning the potential for risk to occur; then a decision is made whether to test at a higher tier. Each tier is more extensive and complex than the preceding one, requires more manpower, and is more costly. The assessment should not proceed if no risk is apparent or if the risk is sufficiently great that action is warranted immediately.

Tier 1 involves a literature study primarily, but also includes historical site information, existing field data, literature and output from fate and effects models, and previous field surveys of T&E or other relevant species. Measurement endpoints rely on available data with conservative assumptions that infer protection for assessment endpoints. These data may be used to develop preliminary hazard indices or risk quotients.

Tier 2 addresses site-specific issues, limiting reliance on values from literature. This may include more models, laboratory tests, or limited field studies to address data gaps in exposure or ecological effects, and use more sophisticated analyses to develop more rigorous hazard indices. Measurement endpoints should be more

complex, based on specific laboratory or filed studies that address data gaps identified in Tier 1.

Tier 3 involves increased complexity, combining site-specific field observations with laboratory and field data to refine exposure and ecological effects characterization. Studies may include population- and ecosystem-level complexity and continue over a longer term. Uncertainty associated with measurement endpoints is reduced.

Each tier incorporates the same steps of evaluation but with increasing specificity. Measurement endpoints will change with each tier, but the assessment endpoints apply to each tier.

It should be noted that, while ERA for T&E and non-T&E species are essentially identical, the level of biological organization that is sought to be protected differs dramatically between the two assessments. Risk assessments for T&E species are a more conservative subset of those performed for non-T&E species. For non-T&E species, the purpose of the risk assessment is to protect *populations*. A level of effect is identified at the outset of the assessment (e.g., a 5, 10, or 20 percent reduction in abundance or reproduction of the endpoint species) that is taken to be representative of an adverse effect on the population of the endpoint species. The focus of all subsequent data collection is to determine if the population is affected by the contaminant or other stressor.

T&E species, by definition, have limited populations. Because of their limited population size, the loss of any *individual* could have a serious adverse impact on the continued survival of the population and possibly of the entire species. Therefore, an ERA for T&E species focuses on adverse effects on the *individual*. Any adverse effect that could affect survival or reproduction that is identified for an individual may be a serious concern.

3 Data Needs for Ecological Risk Assessment

Ecological risk assessment uses both field and laboratory studies to quantify the nature and magnitude of effects. As in human health risk assessment, ERA for T&E species is limited by legal and ethical considerations that preclude investigations that could result in mortality or other adverse impacts to T&E species. However, surrogate species ecologically or taxonomically similar to the T&E species of interest may be used to approximate effects expected on the T&E species.

Effects of smokes and obscurants on T&E plants and animals may be evaluated using a combination of literature-based toxicity data, biological survey data, and ambient media toxicity test data. These data serve to qualitatively and quantitatively assess the relationship between contaminant exposure and direct or indirect effects. Direct effects are lethal and sublethal effects to an individual organism resulting from exposure to a contaminant. Examples of direct effects include mortality, reproductive failure, and reduced growth. Indirect effects are contaminant-induced changes in a species' environment, including altered food availability, interspecies interaction (e.g., competition and predation), and habitat quality and quantity. The primary use of literature-based toxicity data and ambient media toxicity tests is to evaluate direct effects to the endpoint species. These data may also be used to evaluate effects to prey species that may result in an indirect effect on the endpoint species. In contrast, biological survey data may be used to evaluate both direct and indirect effects.

Literature-based Toxicity Data

Literature-based toxicity data are results of toxicity tests conducted in the laboratory on individual chemicals. These data are published as values in the literature or databases and include measures of acutely lethal toxicity and chronic lethal and nonlethal toxic effects on individual organisms. These data may then be used to derive toxicological benchmarks that represent levels of contaminants believed to have no adverse effects on endpoint species. Methods for the development of benchmarks and benchmark values for selected contaminants are available for aquatic biota (Suter and Mabrey 1994), sediment-associated biota (Hull and

Suter 1994), wildlife (Opresko et al. 1994), plants (Will and Suter 1994a), and soil invertebrates and soil processes (Will and Suter 1994b).

In practice, literature-based toxicity data are compared with estimates of contaminant exposure for endpoint species. If exposure exceeds the toxicity value, a hazard may exist. To evaluate exposure of T&E species to smokes and obscurants, data must be obtained on the spatial distribution and magnitude of residues in media to which T&E species may be exposed. Because aquatic, sediment, and soil associated biota are exposed primarily through one medium, the residue concentrations in water, sediment, and soil may be used as simple exposure estimates for these species.

Exposure estimation for birds, mammals, reptiles, and amphibians is more complicated because these taxa may be exposed through multiple routes: orally (i.e., food, water, and soil ingestion), dermally (absorption through skin), and through inhalation (an obvious concern for smokes and obscurants). Generalized models to estimate oral contaminant exposure for wildlife are presented by Sample and Suter (1994). Methods for estimating dermal and inhalation exposure by wildlife are poorly defined; however, a general discussion may be found in EPA (1993a).

A significant limitation to the use of literature-based toxicity data to evaluate risks from smokes and obscurants is the limited availability of published toxicity data. Although exposure estimates may be generated for any T&E species, the estimates are of little value if no toxicity values exist with which to compare.

Biological Survey Data

Biological survey data consist of counts of the abundance, diversity, distribution, and condition of plants, animals, or their habitats. These data provide a measure of the health, abundance, and distribution of T&E species, surrogate species, and prey species. Survey data may also be used to evaluate the availability, quality, and distribution of habitat for T&E species. Biological survey data provide a reality check on the other lines of evidence. For example, if media toxicity tests or literature-based toxicity values suggest that toxic effects should be occurring, but biological survey data show healthy organisms and abundant populations in smoke-exposed areas, validity of the other two lines of evidence should be reevaluated.

Biological survey data are critical in the evaluation of risks to T&E species. Surveys may be used to determine if exposure will occur. For example, areas containing populations of T&E species or their critical habitat can be identified and

compared with areas where smokes and obscurants are used. If these areas do not overlap and are mutually exclusive, exposure of T&E species to smoke residues is unlikely; therefore, risks to T&E species are unlikely. In areas where exposure does occur, biological surveys may be used to estimate effects. In addition, biological surveys may be used to measure the magnitude of exposure by comparing how frequently smokes are used in a given area to the amount of use the area receives from T&E species.

The high degree of natural variation inherent to all biological field data makes it extremely important to concurrently collect data from one to several uncontaminated reference locations when collecting biological survey data for risk assessment purposes. By comparing the data from the reference location(s) with that from the impacted site, effects attributable to smoke and obscurant exposure may be differentiated from population fluctuations or habitat alterations that result from other causes. Selection of multiple reference sites as comparable to the impacted site as possible is highly recommended. If only a single reference site is used, observed differences may indicate site differences and be wholly unrelated to smoke exposure. Use of multiple reference sites greatly reduces the effect of site differences in the risk assessment.

Although differences observed between the contaminated site and uncontaminated reference sites may show the presence and nature of an effect, they do not indicate the cause and source of the effect. Additional data on the toxicity and biological effects of contaminants found at the site are needed. These data are obtained through the use of media toxicity tests and literature-based toxicity data.

Media Toxicity Data

Media toxicity tests are performed by placing test plants or animals in media (soil, sediment, or water) collected from the contaminated site and observing their survival or other responses. The primary strength of these tests is that they are site specific, providing an indication of the toxicity and bioavailability of the combination of contaminants found at a particular site. These tests also provide the real-world link between biological surveys and literature-based toxicity data. For example, if the biological survey data suggests a contaminant effect but comparison of contaminant concentrations at the site with literature-based toxicity data indicates that effects are unlikely, media toxicity tests may serve to confirm or refute whether contamination is a likely cause of the observed biological differences.

Media toxicity tests may be performed either in the laboratory or *in situ*. Because environmental conditions can be controlled and standardized, laboratory toxicity tests allow toxicity to be determined without potentially confounding influences of environmental conditions. Because environmental conditions are standardized, results from laboratory toxicity tests may be compared with tests conducted at different times with media from different locations. However, because they use standard environmental conditions, results obtained may not reflect toxicity that may be observed in the variable conditions at the site. *In situ* toxicity tests, because they are conducted at the site, incorporate and reflect the interaction between toxicity and environmental conditions. However, because of the variability of conditions from site to site and over time, comparisons between tests are more difficult. Ideally, if appropriate methods are available, a combination of laboratory and *in situ* toxicity tests should be used.

4 Approach for Selection of Methods

Before collection of data to assess smoke and obscurant risk to T&E species can begin, appropriate methods for data collection must be identified. A four-step approach for the selection of appropriate sampling methods is outlined in this chapter. This approach consists of identifying the T&E species of concern, identifying the contaminants of potential concern, developing a conceptual model, and selecting appropriate sampling methods based on the results of the first three steps.

Identify T&E Species of Concern

Because of the large number of T&E species that may occur on military reservations and their taxonomic and ecological diversity, identification of these species is by far the most crucial step in selecting appropriate methods of data collection. The choice of appropriate methods, biological survey and toxicity test methods in particular, is highly dependant on the species to be evaluated. For example, methods appropriate to assess risk to plants are likely to be inappropriate for testing endangered mussels.

Once a species has been identified, a literature search should be performed to identify all available information concerning life history requirements (i.e., food habits, habitat requirements, critical habitat, etc.), methods used by researchers investigating the ecology of the species, and persons with experience studying the species. If available, recovery plans for the particular species should be obtained from the U.S. Fish and Wildlife Service (USFWS). These plans will contain information on life history, factors that place populations of the species at risk, and persons to contact for more information. Additional information on endangered species in North America can be found in Lowe, Matthews, and Moseley (1994). Information obtained in this search will aid in the selection of the most appropriate methods.

Identify Contaminant(s) of Potential Concern

Smokes and obscurants can consist of metals, chlorinated hydrocarbons, or oils, in various combinations and may be used as munitions (i.e., grenades or projectiles)

or produced from stationary generators. Impacts may result not only from the smoke material but also from its breakdown products. Exposure and effects of these smokes and obscurants are highly dependant not only on their chemical composition but on their use patterns. Areas where smokes are frequently used are likely to be more impacted than areas where their use is only occasional.

Toxicological effects and environmental fate and transport are functions of chemical characteristics. To select methods appropriate to evaluate environmental residues and estimate exposure, to identify areas or habitats potentially affected, and to select the most appropriate toxicity tests, the following information is needed:

- the chemical composition of the smoke or obscurant
- breakdown products
- bioaccumulation potential
- environmental fate and transport
- toxicity information
- general use practices (i.e., delivery system used, frequency of use, etc.).

Develop Conceptual Model

The primary purpose of a conceptual model in ERA is to develop working hypotheses describing the interaction between a stressor and ecological endpoints (EPA 1992a). A conceptual model graphically represents processes that may adversely affect T&E species. These processes include transport of contaminants on the site, movement of contaminants off the site, uptake by biota (either directly or through food webs), and propagation of secondary effects through ecological interactions. Ecological characteristics of the T&E species of interest are integrated with application and environmental fate characteristics of the smoke compound to develop a conceptual model describing the expected interaction between T&E species and the smoke or obscurant. The primary purposes of conceptual models are to focus the risk assessment on the most important questions as they relate to a particular endpoint-stressor combination and to guide the selection of sampling methods so that data most useful to assess risk are collected.

Figures 1 through 4 are examples of conceptual models for representative T&E species, the red-cockaded woodpecker (*Picoides borealis*), the bald eagle (*Haliaeetus leucocephalus*), the gopher tortoise (*Gopherus polyphemus*), and an endangered plant. The models flow from the source (rounded box at top) to the endpoint (oval box at bottom). Square boxes represent abiotic media critical to contaminant transfer. Air is not specifically identified but is implicitly assumed to be the

primary transport medium for smokes. Diamond-shaped boxes represent the primary biological media with which the endpoint species interacts. Arrows linking the boxes display the relationship between components in the model. Notations adjacent to arrows describe direct toxicity, fate and transport of smokes or their residues, or indirect or ecological effects.

Select Sampling Methods

Guided by the life history characteristics of the T&E species of interest, environmental fate, transport, and use data for the smoke or obscurant, and the contaminant transfer and effects pathways outlined in the conceptual model, appropriate methods may now be selected. To perform a robust risk assessment and to reduce the magnitude of uncertainty, data for all three lines of evidence (literature-based toxicity data, biological survey data, and toxicity test data) should be collected if possible. Media sampling methods for exposure estimation, biological survey methods, and toxicity test methods are presented in Chapters 6, 7, and 8, respectively.

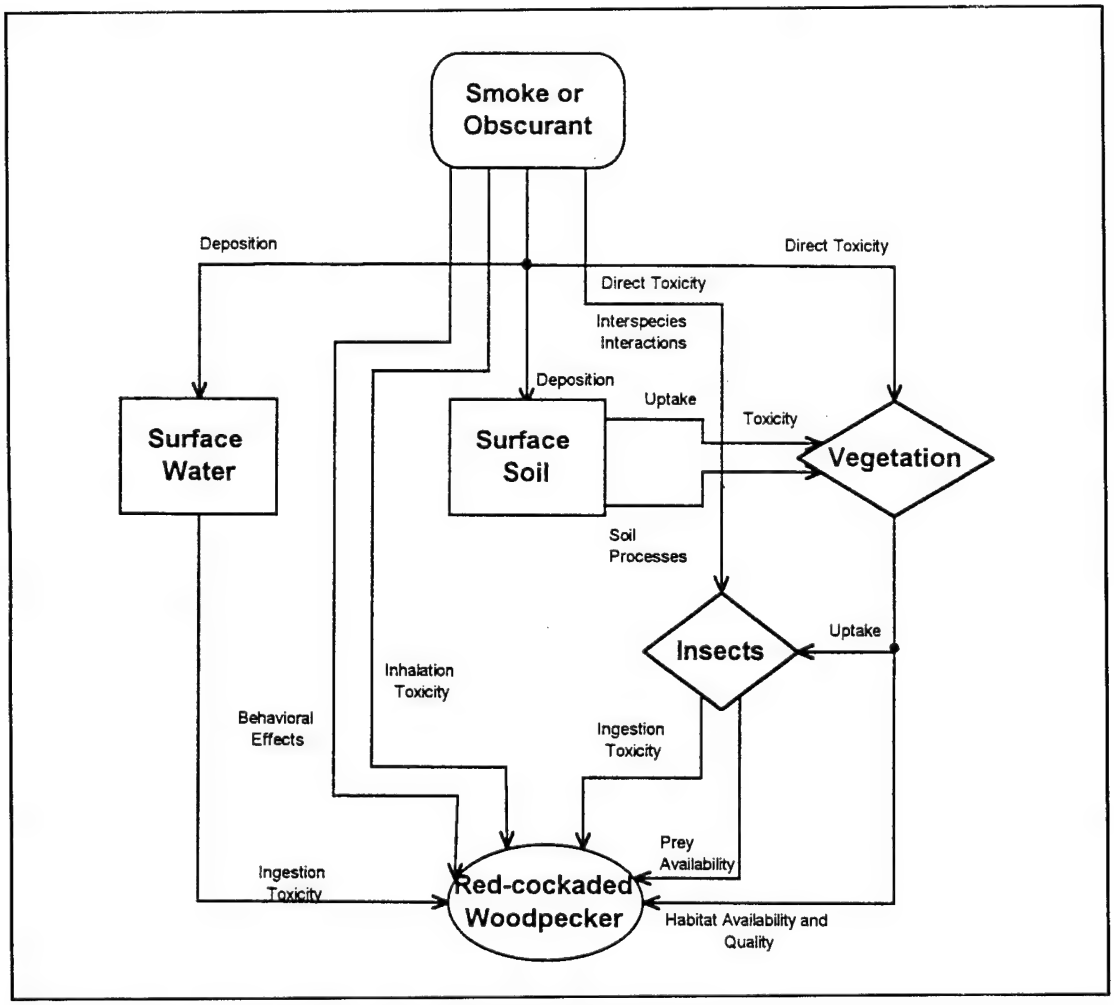

Figure 1. Conceptual model for the red-cockaded woodpecker.

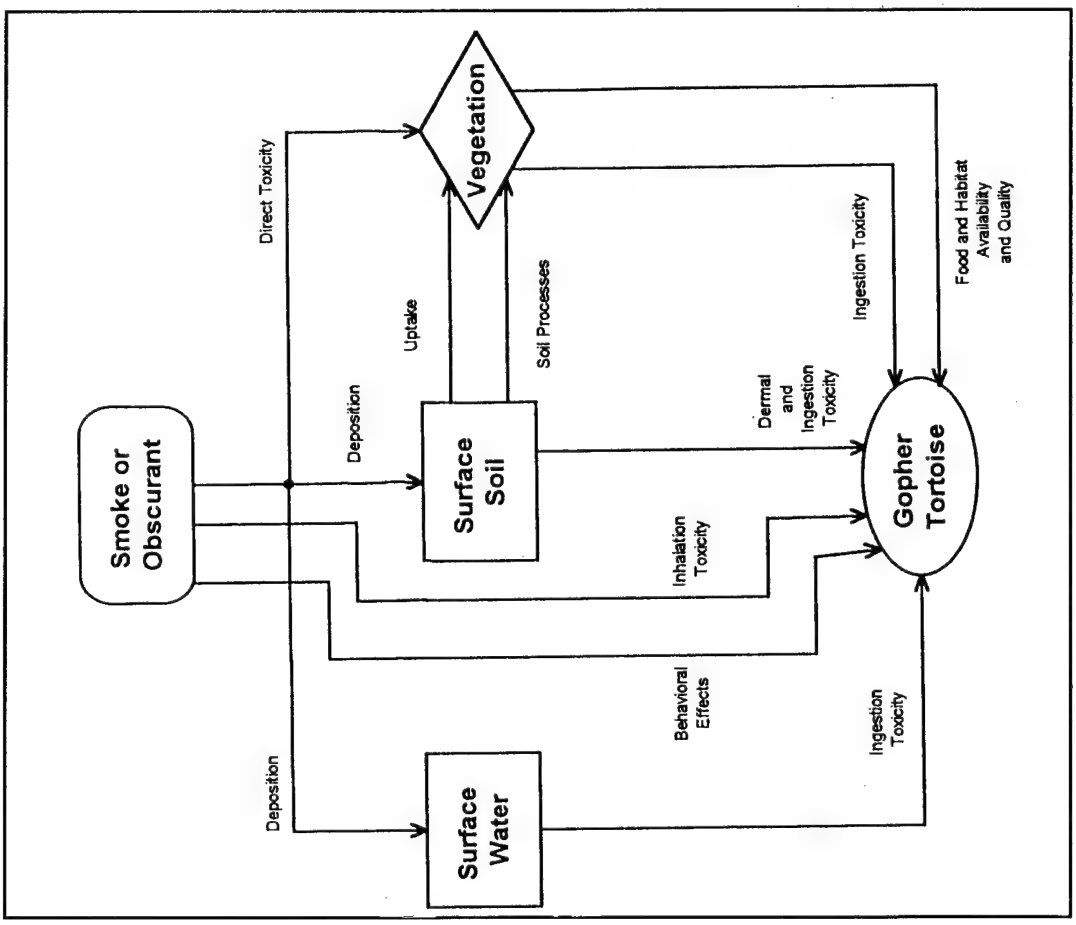

Figure 3. Conceptual model for the gopher tortoise.

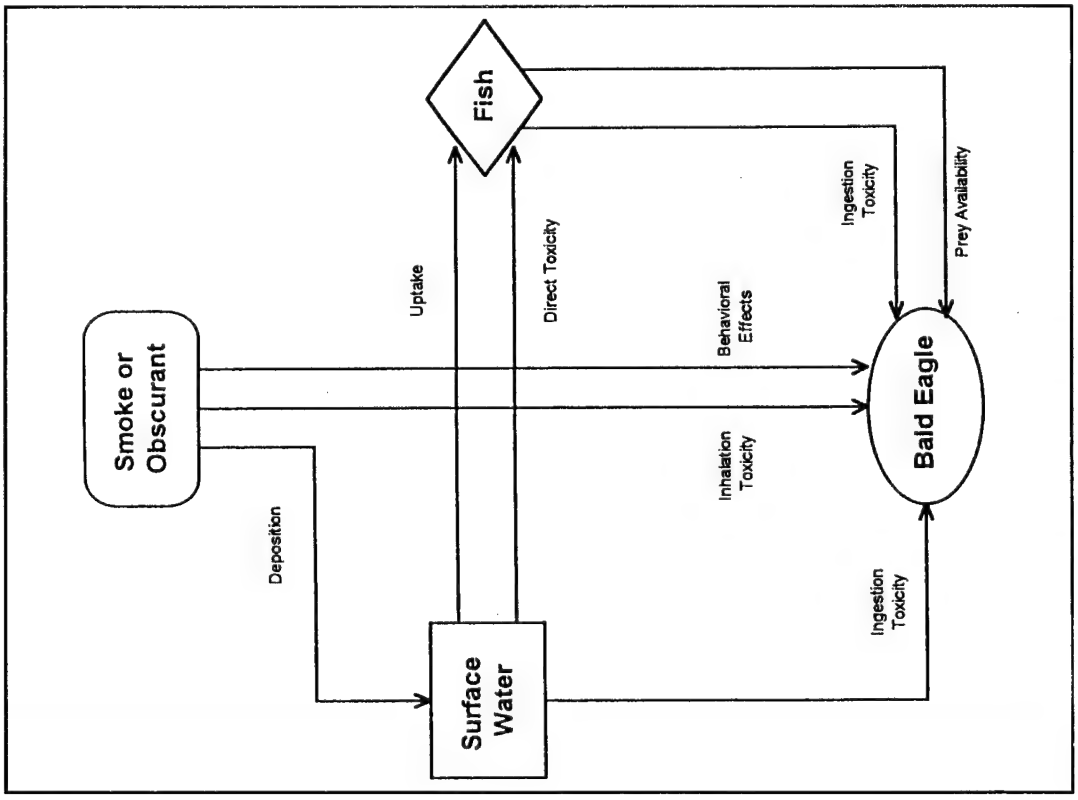

Figure 2. Conceptual model for the bald eagle.

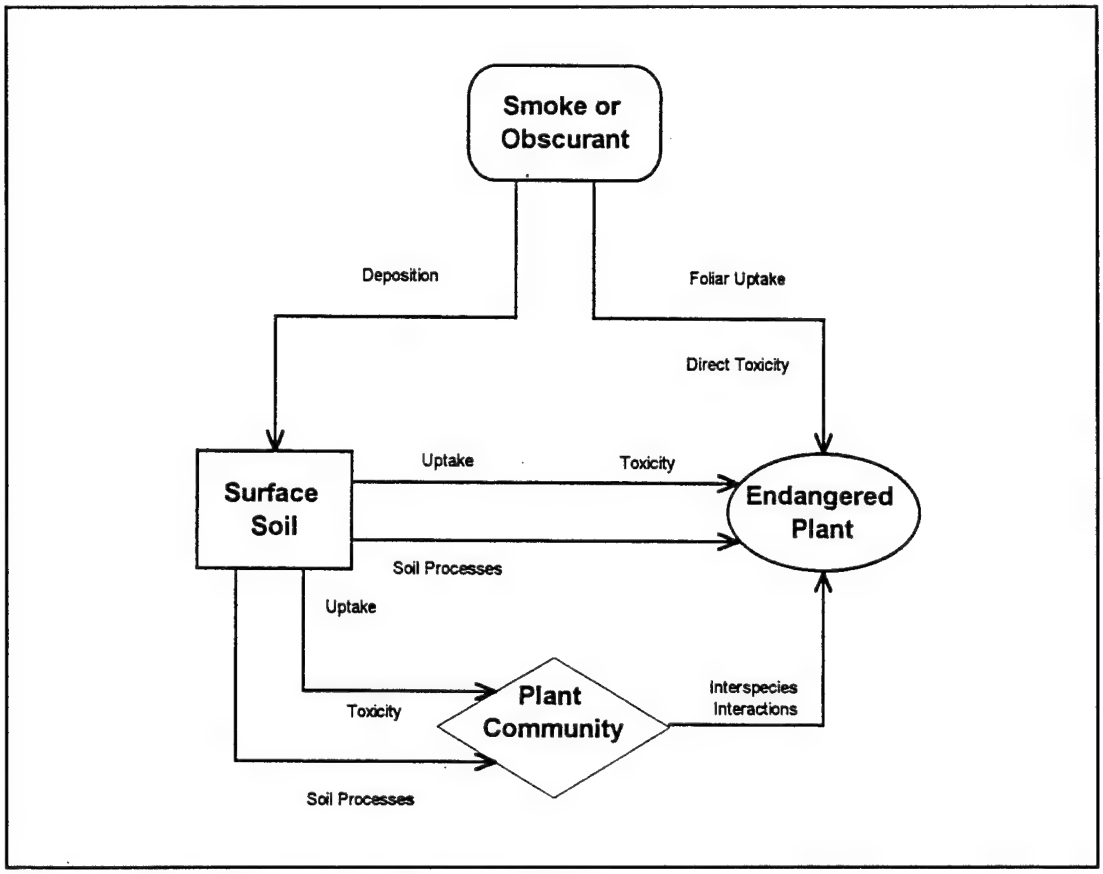

Figure 4. Conceptual model for an endangered plant.

5 Sampling and Analysis Plan

A sampling plan must be developed before initiating any field data collection. Based on pathways and endpoints summarized in the conceptual model (see Chapter 4), the plan outlines the purposes and goals of the sampling program and identifies the equipment, methodologies, and logistics to be used. The plan should be clear, concise, and include the following components (EPA 1983a):

- background information on the problem
- objectives and goals of the field sampling program
- sampling methods to be used, including equipment needs, procedures, etc.
- sample locations (if possible)
- sampling design
- analytical methods
- special permits required (critical in any work concerning T&E species).

These components are intended to guide sampling plan development and are not all inclusive. Additional elements may be added or deleted depending on the specific requirements of the field study.

To ensure that the most appropriate methods are used and that sufficient data to accurately estimate risk are collected, a Data Quality Objectives (DQO) process, comparable to that described in EPA (1993b), should be followed. In a CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act) context, the DQO process is a planning tool to help site managers decide what type, quality, and quantity of data will be sufficient for environmental decisionmaking. The outputs of the DQO process can be used to develop a statistical sampling design and to effectively plan field studies that can stand up to rigorous review. Although the DQO process is primarily intended to guide the collection of abiotic data for exposure modeling, the general approach can also be applied to the collection of biological data. It is important that all interested parties (i.e., regulators, conservation agencies, etc.) be involved in the DQO process. This involvement will both enhance the quality of data collected and ensure that issues of interest to these groups are addressed.

Sampling Design

Before abiotic and biotic data can be collected, spatial and temporal arrangement of samples (e.g., a sampling design) must be identified. The sampling design should be chosen so that the distribution of data that are produced best represent the actual population distribution. Three common sampling designs are random, stratified, and systematic. Excellent, detailed discussions of sampling designs and methods for data analysis are presented in Green (1979) and Krebs (1989).

Random Sampling

Random sampling uses the concept of random probabilities to choose representative sample locations. Random sampling generally is used when little information exists concerning the contamination or site. It is most effective when the number of available sampling locations is large enough to lend statistical validity to the random-selection process.

Stratified Sampling

Stratified sampling involves the division of the sample population into groups based on knowledge of sample characteristics within these divisions. This approach is used to increase the precision of the estimates made by sampling and is most applicable when the contaminant distribution is heterogeneous and clumped or associated with distinct habitats. Stratified sampling is advantageous when contaminant concentration distributions within divisions are more homogeneous than they are between divisions.

Systematic Sampling

Systematic sampling is the collection of samples at predetermined, regular spatial or temporal intervals. It is the most used sampling scheme; but care must be taken to avoid bias. If, for example, periodic variations occur in the material to be sampled, the systematic plan may become phased with these variations.

A systematic plan often results from approaches that are intended to be random. This result occurs because investigators tend to subdivide a large sample area into increments before randomization.

Animal Care and Use Procedures

Any handling of live animals (including any sample collection) must be in accordance with the Animal Welfare Act (7 USC 2131 et seq.) and other applicable Federal laws, guidelines, and policies. In general, these regulations require that all activities involving live vertebrates be reviewed by a properly constituted animal care and use committee. The appropriate staff within individual MACOMs should be contacted for specific guidance on compliance with these regulations.

6 Media Sampling Methods for Exposure Evaluation

To estimate the extent and magnitude of exposure to smokes and obscurants, both abiotic (water, sediment, soil, and air) and biotic media should be sampled and analyzed for residues of smokes or their breakdown products. Analyses of residue body burdens in biota indicate whether residues are taken up and, therefore, transferred through the food web. Exposure by food web transfer may be estimated by relating the residues found in biota to the food habits and diets of T&E species. Exposure estimates may then be compared with toxicological benchmarks to determine if adverse effects are likely.

Residue analyses also delineate the spatial extent of smoke-affected areas. If the areas affected do not include T&E species or their habitat, exposure of T&E species is unlikely; therefore, risk may be presumed to be negligible. The temporal distribution of contamination may be determined by sampling at various time intervals during and following the use of smokes. These data may be used to identify periods when acute effects may occur (i.e., while smokes are still airborne) and to determine the residence time of residues in various media. Repeated sampling in areas where smokes are used may be used to determine the rates at which residues accumulate or degrade. This information may be helpful in delineating the frequency of use in a given area, so that residues do not exceed hazardous levels.

Abiotic Media

Abiotic media to which T&E species may be exposed include surface water, sediment, soil, and air. Sampling methods for these media are defined in the following sections.

Surface Water

Surface water may become contaminated by residues of smokes and obscurants when these compounds are used in areas containing aquatic habitat. Water contamination may consist of residues and unexpended materials deposited on the

water surface or, if the compounds (or their residues) are water soluble, residues dissolved in water. Water contamination may present a hazard to all aquatic biota and to those terrestrial biota that drink from contaminated surface water sources or feed on aquatic biota.

The four primary methods used for collecting surface water samples are sample container immersion, dipper, peristaltic pump, and Kemmerer bottle. Sample container immersion and dippers may be used in any type or size of water body but are best when shallow or surface-water samples are required. Peristaltic pumps also may be used in any size water body and are best for subsurface samples from depths of 0 to 8 m. In contrast, Kemmerer bottles, while being effective for sampling at depth, are applicable only for larger bodies of water. Each method is briefly described in the following sections. Detailed descriptions of the application of these methods and procedures for their use are in EPA (1983a).

Sample container immersion. This is the simplest surface water sampling method. It is applicable for sampling water from shallow streams or the near shore of ponds, lakes, rivers, etc. The sampling vessel is a bottle simply submerged and allowed to fill. This method is advantageous if the sample could be altered by transferring the sample from the collection vessel to another container such as when material may adhere to the inside of the sample collection vessel, resulting in inaccurately low analytical results. A drawback to this method is that the external surface of the sampling vessel is likely to require decontamination. Because the method requires immersion of hands, personnel should wear gloves to prevent exposure to contaminants.

Dipper. A dipper or other container constructed of inert material (teflon or stainless steel) may be used to collect and transfer surface water from the source to the sample container. This method prevents unnecessary contamination of the sample container and sampling personnel. Dippers may consist of ladles or ice scoops available from commercial kitchen or laboratory supply houses.

A modification of this method is the pond sampler, which consists of an adjustable clamp attached to the end of a two- to three-piece telescoping tube that serves as a handle. The clamp is used to secure a sampling beaker. The telescoping handle serves to extend the reach of the sampling technician, allowing samples to be collected at a distance from the shore, bank, or boat. Pond samplers, although not commercially available, may be easily fabricated. Telescoping tubing may be obtained from hardware stores; adjustable clamps may be obtained from laboratory supply houses.

Peristaltic pump. This method is practical for a wide range of applications, including the sampling of streams, rivers, ponds, and lakes. The system consists of a peristaltic pump capable of pumping 1 to 3 L per minute and an assortment of teflon or surgical-grade silicon tubing to extend the suction intake. Battery operated pumps are preferable because they do not require direct current (DC) generators or alternating current (AC) converters.

In practice, the suction intake is immersed in the water at the desired depth with the end of the discharge hose that exits the pump placed in a sample bottle. The pump is turned on, filling the sample bottle.

Peristaltic pumps are portable; samples are collected through essentially chemically inert materials. This procedure can extend the lateral reach of the sample collector, which allows for sampling at depth and across the width of narrow streams. To avoid cross-contamination, tubing should be replaced between sample locations. Sufficient tubing should be allocated to prevent the necessity of cleaning tubing in the field. The primary drawback of peristaltic pumps is that their lift capacity (and therefore sampling depth) is limited to approximately 8 m.

Kemmerer bottle. The Kemmerer bottle is a messenger-activated water sampling device used when discrete samples are required from within the water column at depths greater than can be sampled by peristaltic pumps. In the open position, water flows easily through the device. Once lowered to the desired depth, the release mechanism is tripped, closing the bottle. When closed, the bottle is sealed from additional contact with the water column and can be retrieved. After the bottle has been retrieved, its contents are drained into an appropriate sample bottle. This sample method is applicable only in large bodies of water where samples at depth are required. Cross-contamination between water strata is also a concern with this method.

Sediment

After smokes and obscurants have been used in areas containing aquatic habitats, residues deposited on the water are likely to settle through the water column and deposit in sediment. Residues in sediment may present a risk to T&E fish, mollusks, and aquatic reptiles and amphibians.

The three general types of sediment sampling devices are dredges, grabs, and corers. A dredge is a vessel that can be dragged across the sediment surface, either by a boat or by a handle (Baudo 1990). As it passes over the surface, the dredge digs into the sediment, collecting the surface layer. Dredges are used primarily to

collect benthic fauna and have the advantage of covering a large area, thus producing an "average" sample (Baudo 1990). The actual area and sediment depth sampled are difficult to quantify, however. Because dredges disturb and mix the sediment, they are inappropriate for collecting sediment samples for toxicity tests. In addition, because dredges are made of fabric or have net in the bottom, they act as sieves and do not retain all fine-textured sediment materials or small benthic fauna.

The second sampling device, the grab, generally consists of two metal jaws that can be closed after the device reaches the bottom. The jaws are closed either automatically or following a mechanical or electrical signal from the surface. Grabs are favored by biologists because the size of the area sampled is known, and they can produce large samples of the top layers of sediment, where benthic biota are likely to be found (Baudo 1990). The depth of samples taken with grabs is variable and depends on the density and composition of the sediment and the weight and speed of the grab when it hits the bottom. Sample quality may also be affected by perturbations of the sediment surface during both impact and opening of the grab for sample removal.

Corers, the third type of sediment sampling device, consist of a cylindrical or square tube inserted into the sediment to extract a sample. Corers are designed to provide the maximum amount of sample with the least disturbance of the sediment. Many types of corers exist (Baudo 1990). Small push corers and small gravity corers can be retrieved by hand and used from a small boat. Larger more complicated corers, such as piston or vibrocorers, may require a lifting boom, winch, larger sampling vessels, and larger field crews.

Detailed discussions of sediment samplers and their use are in ASTM (1990), Baudo, Giesy, and Muntau (1990), EPA (1983a), EPA (1994a), Burton (1992), and Mudroch and MacKnight (1991). Table 1 summarizes the advantages and disadvantages of various sediment samplers.

Soil

Most smoke residues are ultimately deposited in the surface soil. Contaminants in soil may present a hazard to all terrestrial plants and most terrestrial wildlife. In addition, erosion of contaminated soils may present a hazard to aquatic biota.

A brief summary of soil sampling methods is provided in EPA (1983a). For an in-depth discussion of soil and soil sampling, consult EPA (1983b), which discusses the factors that influence the selection of sampling schemes and field sampling methods, emphasizing statistical design and data analysis.

Table 1. Advantages and disadvantages of various sediment samplers.

Sampler	Advantages	Disadvantages
Hand and gravity corers	Maintain sediment layering of the inner core. Fine surficial sediments retained by hand corer. Replicate samples efficiently obtained. Removable liners. Inert liners may be used. Quantitative sampling allowed.	Small sample volume. Gravity corer may result in loss of fine surficial sediments. Liner removal required for repetitive sampling. Not suitable for coarse-grain or consolidated sediments.
Box corer	Maintains sediment layering of large volume of sediment. Surficial fine sediments retained relatively well. Quantitative sampling allowed. Excellent control of depth of penetration.	Size and weight require power winch; difficult to handle and transport. Not suitable for consolidated sediment.
Vibracorer	Samples deep sediment for historical analyses. Samples consolidated sediments.	Expensive and requires winch. Outer core integrity slightly disrupted.
Ekman or box dredge	Relatively large volume of sediment may be obtained. May be subsampled through lid. Lid design reduces loss of surficial sediments as compared to many dredges. Usable in moderately compacted sediments of varying grain sizes.	Loss of fine sediments may occur during sampling. Incomplete jaw closure occurs in coarse-grain sediments or with large debris. Sediment integrity disrupted. Not an inert surface.
Ponar grab	Commonly used. Large volume of sediment obtained. Adequate in most substrates. Weight allows use in deep waters. Good sediment penetration.	Loss of fine sediments and sediment integrity occurs. Incomplete jaw closure occurs occasionally. Not an inert surface. Heavy and requires a winch.
van Veen or Young grab	Useful in deep water and on most substrates. Young grab coated with inert polymer. Large sediment volume obtained.	Loss of fine sediments and sediment integrity occurs. Incomplete jaw closure possible. van Veen grab has metal surface. Both may require a winch.
Petersen grab	Large sediment volume obtained from most substrates in deep water.	Loss of fine sediments and sediment integrity. Not an inert surface. Incomplete jaw closure may occur. May require a winch.
Orange-peel grab	Large sediment volumes obtained from most substrates. Efficient closure.	Loss of fine sediments and sediment integrity. Not an inert surface. Requires a winch.
Shipek grab	Adequate on most surfaces.	Small volume. Loss of fine sediments and sediment integrity. Not an inert surface.

*Sources: Adapted from Burton (1992) and EPA (1994a).

Shallow soil samples. In areas where smokes and obscurants are used only occasionally and intermittently, residues are likely to be restricted to the surface soil and litter layers. The surface soil layers are also the most critical in estimating exposure to terrestrial wildlife. Methods for sampling the surface soil layers (approximately the top 15 cm) will therefore be most applicable. Devices for sampling surface soil include the soil punch, ring samplers, or a simple scoop or shovel, depending on the properties of the soil (EPA 1983b).

A soil punch is a thin-walled steel tube 15- to 20-cm long, 2.5-cm in diameter. Driven into the ground with a mallet, the tube is then extracted and the soil within the tube removed. Soil punches are fast, easy to use, and are readily adapted to various analytical schemes.

A ring sampler is a steel ring approximately 15 to 30 cm in diameter that is driven into the soil to a depth of 15 to 20 cm. Cores produced by ring samplers permit results to be expressed on a per unit area basis and allow a constant area of soil to be collected each time. Removal of cores may be difficult in loose, sandy soils or tight clayey soils.

Sample collection using a scoop or shovel is the least desirable method; this method is applicable only if area or volume of the sample are not critical. It is difficult to repeatedly sample to the same depth using this method. Because of the high variability and poor consistency of results, using a scoop or shovel is not recommended.

Deep soil samples. Where smokes have been heavily used for a long time, residues may have migrated to deeper soil horizons than can be sampled using the techniques already described. Contaminants in these deeper layers may present a hazard to burrowing animals and to deep-rooted plants. To evaluate deep soil contamination that may occur in areas where smokes are heavily used, soil augers, power driven corers, or trenching may be used. Descriptions and applications of these methods are in EPA (1983b).

Compositing of samples. Compositing of samples is a common practice in soil analyses. Compositing consists of combining and mixing samples from multiple locations or from various depths to produce one sample. This composite sample reduces the number of analyses required. Although composite samples may accurately represent the mean of the combined samples, information on sample variance is lost. For risk assessment purposes, compositing of samples should be limited to data collected for screening purposes. To assess risk from smokes, samples from various depths should be analyzed separately and should not be composited because surface layers are likely to have greater residues than deeper layers. In addition, soil samples from different locations should not be composited if a study objective is to define the areal distribution of residues in the soil.

Removal of litter. Most locations where soil is to be collected are either vegetated or covered with litter. To differentiate between surface soil and litter contamination, all litter or vegetation should be removed down to the upper humus layer, prior

to the collection of the soil sample. Litter or ground vegetation that is removed should be retained and analyzed for smoke residues.

Air

Because smokes and obscurants are transported and distributed by air, sampling of the air and determination of aerial deposition are critical to the estimation of exposure of biota to these materials (Policastro et al. 1990). Appropriate sampling methods for these smokes and obscurants are in a draft technical report by Cassels and Reinbold (June 1996). Cassels and Reinbold's report will be Volume 3 of this technical report series.

Biotic Media

To evaluate the risk presented by food-web transfer of smoke residues, biota that may be consumed by T&E species should be sampled and analyzed. Methods for collecting biotic samples are summarized in the following sections. It should be noted that residue analyses frequently require the destruction of the sample. For this reason, T&E species should not be sampled for residue analyses.

Plants

In areas where smokes are used, plants may accumulate residues either through root uptake from the soil or uptake of residues deposited on foliage. Smoke residues taken up by the plant or deposited on foliage may then put herbivorous T&E species at risk. In addition, uptake by and exposure of T&E plant species may be approximated by sampling and analyzing plants that are taxonomically similar to the T&E plant species.

With the exception of some trace metals, such as arsenic and selenium, uptake of contaminants by plants from soil is unlikely to be a major contaminant pathway. However, particulate fractions of smokes and obscurants can be deposited on plant parts (e.g., leaves, stems, and fruit) either directly or through precipitation or resuspension of contaminated soil. In addition to direct damage caused by thermal, pyrophoric, or caustic properties of these contaminants, deposition may impact the plant by interfering physically or chemically with physiological processes such as photosynthesis or respiration.

Contaminants incorporated into plant tissues through uptake or deposited on exposed plant parts are potentially available to plant consumers and the rest of the

terrestrial food chain. Thus, T&E wildlife of all trophic levels may be at risk. Models to predict uptake of contaminants by plants are not well developed for many contaminants and are virtually nonexistent for smokes and obscurants. Moreover, no models are available for reliably predicting the exposure of plants or wildlife to deposited contaminants from smokes and obscurants. For these reasons, it will be essential to measure contaminant concentrations in surrogate or representative plant species for most ERAs involving either T&E plants or T&E herbivores.

Collection of plant material for residue analyses is essentially simple. After plants of the appropriate species are identified, they may be sampled either as whole organisms (roots plus aboveground parts) or as discrete parts (roots, foliage, seeds, fruit, etc.). Samples may be collected by stripping or breaking parts from the plant, by cutting plant parts with shears, or by digging up plants with a spade. If cutting or digging tools are required, tools should be washed between samples to prevent cross-contamination of samples. Sufficient mass should be collected for each field sample to provide the analytical laboratory with recommended sample weights. Samples should be collected with as little disturbance as possible to avoid inadvertent loss of deposited contaminants. Samples should also be handled as little as possible; field crews handling the actual tissues should wear plastic gloves to prevent exposure to contaminants.

Once samples have been collected, they should be prepared, stored, and preserved for analysis. In most instances, no preparation is necessary; samples may simply be placed in the sample container. If plants are sampled whole or if root samples are taken, all soil should be washed from the roots using deionized water to prevent cross-contamination. Foliage samples should not be washed if the intended use of the sample is to estimate exposure to herbivores, because washing will remove airborne smoke residue deposits, which may contribute significantly to herbivore exposure. However, if analysis is intended to identify residue uptake by the plant, residues should be washed off.

The method of storage and preservation depends on the type of analysis to be performed. The analytical laboratory should be consulted before sample collection to ensure that the appropriate sample containers and preservatives are used.

The primary concern in vegetation sampling is identifying the appropriate species and plant parts for collection. Because herbivores generally do not consume the entire plant and different plant parts accumulate contaminants to different degrees, to evaluate exposure to a T&E herbivore, only those parts that are consumed should be sampled and analyzed. Plant parts sampled should reflect the food habits of the

T&E species of interest. Additional information on vegetation sampling for residue analysis may be found in DOE (1987) and Temple and Wills (1979).

Birds

To evaluate the accumulation of smoke residues by birds and to estimate exposure for T&E species that consume birds (e.g., peregrine falcon), birds may be sampled and analyzed. Capture methods outlined in the following may also be used to catch T&E birds to collect feathers for analysis (Burger 1993) or to facilitate the attachment of radiotransmitters. (Radiotelemetry data for T&E species may be used to estimate the use of areas where smokes are released.) Methods to collect birds include firearms, baited traps, cannon nets, mist nets, drive and drift traps, decoy and enticement lures, and nest traps (Schemnitz 1994). Methods used depend on the species to be sampled.

Permits. Before initiating any sampling program for birds, *all* appropriate permits must be obtained. Taking of migratory waterfowl requires a USFWS permit or a state hunting license (in season) and a Federal waterfowl stamp. Any activity involving T&E species requires a permit from the USFWS and/or the responsible state conservation agency. Permits for the collection of neotropical migratory birds must also be obtained from the USFWS.

Methods. One of the most obvious collection methods is the use of firearms. Firearms used may include rifles, shotguns, or pellet guns. This method, while highly dependant on the skill of field personnel, may be used for all groups of birds. However, because samples may be extensively damaged during collection, projectiles or shot may interfere with residue analyses, and because of safety considerations, the use of firearms is not a recommended sampling method.

Baited traps are most useful for gregarious, seed-eating birds. In their simplest form, a wire-mesh box is supported at one side by a stick, over bait (generally seeds or grain). Once birds enter the box to feed on the seeds, the operator pulls a string attached to the support stick, the box falls, and the birds are entrapped. Other types of baited traps include funnel or ladder traps, which are designed with entrances through which birds can enter easily but not easily exit.

Cannon nets may be used for birds that are too wary to enter traps. This type of trap is frequently used for wild turkey and waterfowl and has been used successfully for sandhill cranes and bald eagles (Schemnitz 1994). Cannon nets consist of a large, light net that is carried over baited birds by mortars or rockets. Nets are

laid out and baited for 1 to 2 weeks to allow the birds to become acclimated to the net and bait. Once birds make regular use of the bait, the trap may be deployed.

Mist netting is a method useful for some species that are not attracted to baits. A detailed review of the use and application of mist nets is provided by Keyes and Grue (1982). This method may be used for birds as large as ducks, hawks, or pheasants but is most applicable to passerines and other birds under ~200 g. Mist nets are constructed from fine, black silk or nylon fibers, usually 0.9 to 2.1 m wide by 9.0 to 11.6 m long, and attached to a cord frame with horizontal crossbraces called "shelfstrings" (Schemnitz 1994). The net is attached to poles at either end so that the shelfstrings are tight, but the net is loose. The loose net hangs below the shelfstrings, forming pockets. When properly deployed, birds (or bats) strike the net and become entangled in the net pocket.

Mist nets may be used passively or actively. In a passive deployment, nets are set across flight corridors and birds are caught as they fly by. For an active deployment, a group of nets is set and birds are driven toward the nets. Another effective approach is to use recorded calls or distress calls of conspecifics to attract birds to the net.

The following must be considered when using mist nets:

- Avoid windy conditions; wind increases the visibility of the net.
- Check nets frequently. Unintended mortality may result from stress if birds are left in the net for more than 1 hour.
- Do not operate nets during rain. Birds may become soaked and may die from hypothermia.
- Special permits are required to use mist nets for migratory birds. These permits must be obtained from the USFWS.

Drive and drift traps are nets or low wire mesh fencing erected at ground level. Birds are driven or herded into the fence, which then guides them into an enclosure. This method is most frequently used to capture waterfowl while they are molting and flightless. Drift traps have also been used successfully with upland gamebirds, rails, and shorebirds (Schemnitz 1994). Because many birds are reluctant to flush and fly when birds of prey are present, trapping success may be enhanced by playing recorded hawk calls.

Decoy and enticement lures are used most frequently for birds of prey. The most common trap of this type is the bal-chatri trap, which consists of a wire mesh cage with numerous monofilament nooses attached to the top. A small bird or rodent is

placed in the trap as bait. When a hawk or owl attempts to attack the bait, the bird of prey becomes entangled in the nooses.

Nest traps are useful to capture birds at the nest for reproductive studies. For ground-nesting birds, drop nets erected over the nest are sometimes effective. For cavity nesting birds, trip doors may be devised that can be closed once the adult enters the nest. Other types of nest traps are summarized by Schemnitz (1994).

Additional information concerning methods for capturing birds may be found in Schemnitz (1994), USFWS and Canadian Wildlife Service (1977), Addy (1956), and Bub (1990).

Euthanasia. Although most capture techniques described for terrestrial vertebrates are designed to capture animals alive, animals generally must be sacrificed before preparation for contaminant residue analysis. (An exception is fur or feather residue analysis, which may be performed on live animals.) It is essential that humane euthanasia methods be used to sacrifice animals for analysis. A detailed discussion of euthanasia methods for birds is presented in Gullet (1987); these methods are adaptable for mammals also.

Euthanasia may be achieved using either physical or chemical methods (Gullet 1987). Physical methods include cervical dislocation, decapitation, stunning and exsanguination, and shooting. Chemical methods include lethal injection or inhalation of anesthetic or toxic gas. Questions to consider when choosing a technique include:

- Will it interfere with residue analyses? (Chemical euthanasia may confound results and may not be recommended.)
- Is it appropriate for the size and type of animal?
- Does it present a risk to human health and safety?
- Is specialized equipment or training required?
- Is it time and cost effective?
- Will the technique offend the casual observer?

Mammals

Many mammalian herbivores and omnivores are prey for T&E wildlife species. In such instances, it may be important to measure the concentration of smoke and obscurant residues that are present in these prey species. For the purposes of this report, this section will focus on sampling methods for three general types of

mammalian prey species: small mammals (i.e., mice, voles, and rats), lagomorphs (i.e., rabbits and hares), and omnivores (i.e., muskrats, opossums, and raccoons).

Permits. In many states, collection of large numbers of small mammals and lagomorphs requires special collection permits available from the state wildlife agency. Check with that agency to learn what permits are required. All states regulate the collection of furbearing species, such as muskrats, and game mammals, such as deer. Again, check with the state wildlife agency to determine what permits are required. These contacts can serve another important purpose. Most state agencies have individual biologists who are responsible for various categories of wildlife. These individuals can be very helpful in designing a sampling program. The furbearer specialist, for example, may be able to provide helpful tips on collection of muskrats.

Small mammals. Collection of small mammals for residue analysis is appropriate if the conceptual model indicates that these animals are important as a source of food for the T&E species of concern. Virtually all predators of small mammals consume the animals whole, so sampling of individual organs is not needed. Small mammals must be analyzed whole, which requires sacrifice of the animal.

Currently, one concern among field researchers and analytical laboratories is that of hanta virus, which occurs in populations of certain small mammals species. Because our knowledge about this virus is growing rapidly, it is impractical to provide guidance on proper protective measures here. Contact the Centers for Disease Control (CDC) in Atlanta (Jim Mills, Pathogens Branch, CDC; telephone: 404-639-1115) to receive their latest information on reasonable measures. Also, contact your analytical laboratory before doing any field work to find out if they require samples to be packaged or shipped in a specific way to protect their workers. Some laboratories insist that any small mammal samples be certified by CDC as being free of hanta virus.

It is important that the conceptual model of the food chain include as much information as possible on what small mammal species are actually used as prey by the T&E species of concern. In most cases, all small rodents may be grouped together and treated as one sample. In other instances, shrews and other insectivores may need to be treated separately. This information is important in selecting the collection method.

Three different types of traps are typically used for small mammals. Box live traps (e.g., Sherman traps), snap traps, and pitfall traps are all effective for small

mammals and shrews (Schemnitz 1994). None of these traps allow for discrimination among species trapped, though shrews are caught less frequently in live traps.

Box live traps allow the field team to decide if a captured animal should be set free or collected, which may be important if there is a high probability that nontarget species may be collected. Of course, live traps then require euthanasia of target animals (see Chapter 6, ***Birds, Euthanasia***). Killing traps eliminate the need for euthanasia, but increase the likelihood of killing nontarget animals if they are present.

Pitfall traps may be either live traps or killing traps (Schemnitz 1994). Adding water or a preservative such as ethylene glycol to a pitfall trap makes it a killing trap. Snap traps are always killing traps. For a review of advantages of pitfall and snap traps see Schemnitz (1994).

Lagomorphs. Although rabbits and hares are not usually consumed entirely by their predators, most tissues are eaten. Thus, it is necessary to sacrifice the animal to sample all relevant tissues (e.g., internal organs, muscle, and fur) to estimate the total exposure to T&E predators.

Rabbits can be collected in at least three ways. Firearms are probably the least desirable method. The possibility of destroying or contaminating important tissues with the projectiles or shot is high with an animal as small as a rabbit. Safety is also a concern with the use of firearms, and the success of the sampling program depends on the skill of the hunter.

Snares are effective means of capturing rabbits. Placed across rabbit pathways, wire snares contract around the animal when it steps through the noose. Struggling by the animal further constricts the noose. Trap mortality with snares is generally high, unless they are visited frequently. Also, snares can capture nontarget small mammals such as mink, weasels, and feral cats.

Cage live traps are also effective for rabbits. These traps are similar in concept to box live traps for small mammals and give the field team the option to release nontarget animals.

Lethal traps, such as conibear traps, are not recommended for lagomorphs because of the high likelihood that nontarget animals will be killed.

Omnivores. Certain large T&E predators (e.g., Florida panthers and red wolves) feed on raccoons. River otters and alligators feed on muskrats. Thus, where these

T&E species exist, trapping of mammalian omnivores may be required. As with rabbits, predators generally consume most edible tissues of raccoons and muskrats, so the sampling program must include all major tissues, and the animals must be sacrificed.

At least two health concerns arise when trapping raccoons. Anyone handling wild omnivores or carnivores should receive the preexposure series of rabies vaccinations. Also, raccoons are hosts to a round worm (*Baylis asceris procyonis*) that can be fatal to humans. This means that field personnel working with raccoons should wear protective gloves and should wash their hands thoroughly after contact with raccoons.

Raccoons and muskrats can be captured in cage live traps (e.g., Hav-a-hart®). Raccoons will respond to many different types of baits, but muskrats tend to respond best to vegetable matter.

Leghold traps and leg snares are also effective for raccoons (Schemnitz 1994). There are concerns relative to animal welfare when using leghold traps, however. Lethal traps should be avoided for raccoons and muskrats, unless the traps can be placed where nontarget animals are unlikely to be caught.

Reptiles and Amphibians

Reptiles and amphibians may represent significant contaminant transfer pathways because they are important prey for some T&E species. Methods suitable for the collection of reptiles and amphibians for residue analysis are summarized in Chapter 7. Additional methods are described in detail in Heyer et al. (1994) and Jones (1986). Most predators are likely to consume all tissues of their prey, so whole-body residue analysis is preferred. For this reason, either lethal or nonlethal collection methods are acceptable. Nonlethal methods permit greater discrimination in specimens collected and reduce the likelihood of collecting nontarget species. Specimens must be euthanized before analysis if nonlethal methods are used. Euthanasia methods for amphibians are discussed in Heyer et al. (1994). In addition, methods of euthanasia for birds or fish may be applicable.

Terrestrial Invertebrates

Terrestrial invertebrates (i.e., mollusks, annelids, arthropods, etc.) are important food resources for many vertebrate species. T&E species may be impacted by smokes via invertebrates in two ways: (1) they may be directly toxic to invertebrates, resulting in reduced abundance (and therefore reducing food available for

T&E species) or (2) they may be accumulated by invertebrates and passed on to T&E invertebrate predators. Methods for sampling of terrestrial invertebrates described in the following sections, while suitable for collection of samples for residue analysis, may also be applicable for population estimation, if samples are collected using a statistically valid design (see Chapter 5, **Sampling Design**).

Mollusks. Methods for the collection of terrestrial mollusks (snails and slugs) are not as well defined as those for other terrestrial invertebrates. Collection methods include the use of bran- or metaldehyde-baited traps or refuge traps (boards placed at a site to attract slugs; Newell 1970). Snails or slugs may also be extracted from litter or soil collected from the site. Snails will generally float and slugs sink when the samples are immersed in water. Although population estimates of snails may be made by counting their abundance within randomly placed quadrats, this method is likely to be biased towards adults and against immatures (Newell 1970). Additional discussion of sampling and extraction of terrestrial mollusks may be found in Newell (1970) and Southwood (1978).

Earthworms. The primary methods for collecting earthworm samples are handsorting of soil, wet sieving, flotation, and the application of expellants.

Regarded as the most accurate sampling method, handsorting is frequently used to evaluate the efficacy of other methods (Satchell 1970; Springett 1981). While accurate, handsorting is very laborious and may underestimate the abundance of small individuals. Efficiency depends on the density of the root mat, clay content of the soil, and weather conditions (if sorting is done in the field).

Wet sieving uses a water jet and a sieve to separate earthworms from the soil (Satchell 1970). While efficiency of this method is not documented, its drawbacks include damage to worms during washing.

Flotation is another water-extraction method (Satchell 1970). Soil samples are placed in water, and earthworms are collected as they float to the surface. This method may be used to extract egg capsules and adults of species too small to recover efficiently by handsorting.

In contrast to methods that require excavation and processing of soil, expellants are applied *in situ* to collect earthworms. In practice, an expellant solution is applied to the soil surface and allowed to percolate down. Earthworms are then collected as they emerge from the soil. To enhance absorption of the expellant by the soil and to facilitate collection of earthworms as they emerge, vegetation at each sampling location should be clipped down to the soil surface.

Expellants have traditionally consisted of formaldehyde or potassium permanganate solutions (Satchell 1970, Raw 1959). Drawbacks to these expellants include carcinogenicity, phytotoxicity, and toxicity to earthworms. In addition, these expellants also may introduce additional contamination and interfere with residue analysis. As an alternative, Gunn (1992) suggested the use of a mustard solution as an expellant. A commercially available prepared mustard emulsion was mixed with water at a rate of 15 mL/L and applied to soil within a 1-m^2 frame (to confine the expellant). Efficacy of mustard was found to be superior to formaldehyde and equivalent to potassium permanganate (Gunn 1992). Recent work at Oak Ridge National Laboratory indicates that dry mustard (1 tsp/L) is also an effective expellant (B. Sample, personal observation). If worm samples are being collected for residue analysis, analyses should be performed on samples of the mustard expellant. These data will indicate if any contamination can be attributed to the extraction method.

Arthropods. Many methods are available to sample terrestrial arthropods. Because of the great diversity of life-history traits and habitats exploited by arthropods, no single method is efficient for capturing all taxa (Julliet 1963). Every sampling method has some associated biases and provides reliable population estimates for only a limited number of taxa (Kunz 1988a; Cooper and Whitmore 1990).

Reviews of sampling methods for insects and other arthropods were given by Southwood (1978), Kunz (1988a), Cooper and Whitmore (1990), and Murkin, Wrubleski, and Ried (1994). Table 2 describes 12 commonly used methods, arthropod groups for which they are appropriate, and summarizes advantages and disadvantages of each.

Benthic Macroinvertebrates

The use of body-burden analysis to determine exposure of T&E benthic macroinvertebrates to smoke residues requires sacrifice of the animals. This is very undesirable, so it is often better to approximate exposure levels by sampling related or surrogate species having similar life histories and habitat requirements. Collection and residue analysis of non-T&E benthic macroinvertebrates may also be used to estimate exposure to those T&E species that consume benthic macroinvertebrates.

Many techniques are suitable for the collection of benthic macroinvertebrates for exposure evaluation. Several such methods are described in the following sections, including *in situ* exposure of a surrogate species maintained in a holding device.

Table 2. Comparison of common arthropod sampling techniques.

Method	Method Description	Arthropods Sampled	Advantages	Disadvantages
Sticky Trap	Adhesive material applied to a surface, usually cylindrical. Arthropods adhere to surface upon contact.	Flying or otherwise active arthropods	Simple, inexpensive, versatile, and portable.	Messy. Temperature affects adhesive. Adhesive likely to interfere with residue analysis. Removal of samples from adhesive difficult, requires use of hazardous chemicals. Quantification of area sampled difficult.
Malaise Trap	Fine mesh netting 'Tent' with baffles that guide arthropods into a collection jar that may or may not contain a killing compound/preservative.	Primarily flying arthropods; crawling arthropods to a lesser degree	Versatile and simple to use. Samples suitable for residue analysis (depends on use of preservative).	Expensive and bulky. Catch strongly affected by trap placement. Biased against Coleoptera. Fewer catches per unit time. Quantification of area sampled difficult.
Shake-cloth	Cloth or catch basin placed beneath plant. When plant is beaten or shaken, arthropods drop onto sheet and are collected.	Foliage-dwelling arthropods	Simple, fast, and easy to perform. Requires minimal equipment. Samples suitable for residue analysis.	Biased against active arthropods and individuals that adhere tightly to vegetation. Quantification of area sampled difficult.
Sweep Net	Among most widely used methods. Insect net is swept through vegetation in a predetermined manner.	Foliage-dwelling arthropods	Simple, fast, and easy to perform. Requires minimal equipment. Samples suitable for residue analysis.	Sample efficacy highly dependant on vegetation structure and sampling personnel. Biased against arthropods that adhere tightly to vegetation. Quantification of area sampled difficult.
Pitfall Trap	Cup or bucket (covered or uncovered) buried in ground up to rim. May or may not contain killing compound/ preservative. May be used with drift fences.	Ground/litter arthropods	Simple and inexpensive. May estimate population density using mark-recapture. Samples suitable for residue analysis (depends on use of preservative).	Biased against inactive arthropods. Very active individuals may escape. Captures affected by density and type of ground cover.
Light Trap	Light source (generally ultraviolet) attached to vanes and a collecting bucket. May or may not use killing compound or preservative.	Nocturnal, phototactic, predominantly flying arthropods	Portable. Simple to use. Collects many taxa, but Lepidoptera predominate. Samples suitable for residue analysis (depends on use of preservative).	Catch affected by environmental conditions and trap placement. Species-specific responses to light unknown. Area sampled cannot be quantified.

Table 2. Continued.

Method	Method Description	Arthropods Sampled	Advantages	Disadvantages
Pesticide Knockdown	Pyrethroid insecticide applied to vegetation by a fogger. Arthropods killed are collected on drop sheets.	Foliage-dwelling arthropods	Simple, fast, and easy to perform. Samples many arthropods with approximately equal probability.	Foggers, pesticides expensive. Affected by wind. May miss extremely active or sessile arthropods. Pesticide may interfere with residue analysis. Quantification of area sampled difficult.
Emergence Trap	Conical or box shaped traps erected over water or soil to collect emerging adult arthropods.	Arthropods emerging from soil or water	Inexpensive. Simple to use. Can estimate density of emerging arthropods. Samples suitable for residue analysis.	Large number may be needed to accurately estimate population.
Pole Pruning	Foliage samples clipped; arthropods on foliage manually removed and counted.	Foliage arthropods (especially Lepidoptera larvae)	Inexpensive and easy to perform. Good for inactive and tightly attached arthropods. Population density can be calculated. Samples suitable for residue analysis.	Biased against active arthropods. Few arthropods per sample. Sample processing is labor intensive.
Portable Vacuum Samplers	Uses portable, generally backpack mounted vacuums to sample insects (Dietrick et al. 1959). Widely used to sample agricultural pests.	Foliage arthropods	Easy to use. Population density can be calculated. Samples suitable for residue analysis.	Expensive (>$1000 each). Best suited for low vegetation; application in forest is questionable. May not accurately sample all taxa.
Stationary Suction	Consists of fan that pushes air through a metallic gauze filter to remove insects (Johnson and Taylor 1955).	Flying arthropods	Easy to use. Population density can be calculated. Samples suitable for residue analysis.	Expensive. Not very portable. Use limited to areas with electrical power. Difficult to sample large areas.
Tree Bands	Burlap bands are attached to trees. Takes advantage of tendency of some arthropods to move vertically on tree trunks.	Vertically mobile arthropods	Simple and inexpensive. Population density may be calculated. Samples suitable for residue analysis.	Installation is time consuming. Biased against most flying species.

Note: Information obtained from Murkin et al. (1994), Cooper and Whitmore (1990), Kunz (1988a), and Southwood (1978) unless otherwise stated.

Benthic organisms can be collected from deep water by divers equipped with snorkels and fins or self-contained underwater breathing apparatus (SCUBA) gear (see Miller et al. 1993 for a description of appropriate safety equipment). This procedure is most amenable for collecting organisms either by hand or with a suction-type sampler. This method would be unsuitable, however, for situations where human exposure to contaminants is a concern.

Suction samplers vacuum the substrate and associated fauna from the streambed into a mesh collection bag, which is removed and taken to the boat or shore for processing. Surrogate organisms can be sieved from the vacuumed material with the aid of a box screen. Although these suction samplers allow rapid and efficient collection of many types of organisms from various substrate types, if T&E species are present they may be collected unintentionally.

Grab samplers (i.e., Ekman, Petersen, Ponar, and Smith-McIntyre samplers) can also be used to collect organisms from deep-water habitats. These devices engulf a portion of substrate (and its associated organisms), which is then hauled to the surface for processing. Organisms are separated from the sample material by washing the substrate in a box screen.

Isom (1978) reviews several types of grab samplers, their specifications, the type of substrate each was designed for, and advantages and disadvantages associated with each type. Grab samplers generally require a boat equipped with a winch and at least two individuals for operation (Isom 1978). A disadvantage of grab samplers is incomplete jaw closure, which can result in loss of part or all of a sample during retrieval (EPA 1973). Furthermore, because grab samplers cover only a small area, several samples may be needed to obtain a suitable number of organisms.

Brails, also called crowfoot bars, consist of a series of four-pronged hooks attached to a bar by short lines or chains, and have been extensively used by researchers and commercial fishermen to collect mussels from large rivers (Bates and Dennis 1985; Starrett 1971; Coker 1919). Collection entails dragging the brail behind a boat and in contact with the stream or lake bed. When a hook passes through the opened shell of a feeding mussel, the mussel reacts by closing its shell on the hook. The brail is then brought up to the boat, where the mollusks are removed. This technique tends to collect larger mollusks, but is moderately nonselective to species. Thus it could unintentionally collect T&E organisms. A complete description of brailing techniques can be found in Starrett (1971) and Coker (1919).

Perhaps the best method for collecting slow-moving or large sessile organisms is hand-picking while wading in shallow water. Organisms are located by sight and simply removed from the substrate.

A d-frame net or kick net may be used to collect smaller and more mobile species in shallow streams. The net is placed against the streambed, and the substrate upstream of the mouth of the net is agitated to suspend the organisms, which are then washed into the net by the current. Appendix A gives further details of this collection technique.

Coring devices can be used in both shallow and deep water to sample invertebrates. These devices are inserted into the substrate and provide a sample of substrate and organisms. The sample is washed in a sieve and the organisms are removed from the remaining sample debris. Smock et al. (1992) and Williams and Hynes (1973) give in-depth information on core sampling.

Peterson and Southworth (1994) describe a method to estimate *in situ* exposure using surrogate species. The selected organisms are held in polypropylene cages that are placed in the area of potential contamination and each reference site. After the prescribed period of exposure (generally 4 weeks), the organisms are analyzed for contaminants and levels are compared with those at the reference sites. Indigenous organisms should be used whenever possible to prevent the unintentional introduction of exotic species where they do not exist.

Fish

Fish in areas in which smokes and obscurants are used may be exposed to residues through uptake from the gills, accidental ingestion of contaminated sediments, or by accumulation through the food chain. Smoke residues accumulated by fish then provide a contaminant transfer pathway to piscivorous wildlife.

Sampling techniques for fish include seines and mobile nets, electrofishing, stationary nets and traps, visual observations, and chemicals. These methods may be used both to collect samples for residue analysis and to survey populations of T&E and non-T&E fish species. The applications, advantages, and disadvantages of these methods are discussed in detail in Chapter 7.

Fish sampled for body burden analysis must be euthanized before analysis. Common methods include the use of a chemical anesthetic agent or cold anesthesia. Most chemical anesthetics provide rapid and effective euthanasia. Chemical

anesthetics should be used with caution, however, because they may interfere with and confound residue analysis.

Cold anesthesia, another quick and effective method, consists of placing fish in coolers of ice. Dry ice may also be used, if tissue freezing is desired. Cold anesthesia avoids any possible contamination of tissues by the chemical anesthetic. Reviews of anesthetics and handling techniques are given in Stickney (1983) and Summerfelt and Smith (1990).

To evaluate exposure experienced by T&E fish species, media to which they are exposed (e.g., water, sediment, and food) should be sampled and analyzed. Sampling of surface water and sediments are covered earlier in the **Abiotic Media** portion of this chapter. These methods should be used to sample the media directly associated with the critical habitat of the T&E species. Food chain effects may be analyzed by sampling the principal food items of the target T&E species. If the food items are not identified in the literature, it should be possible to use pumps or flushing devices to nondestructively sample stomach contents (Baker and Fraser 1976, Bowen 1983). Another approach would be to analyze stomach contents of a surrogate species. The surrogate species should be as similar taxonomically as possible or be a species similar in feeding habits (e.g., a benthic insectivore) and habitat requirements. If an indication of the possible accumulation of the chemicals is desired, the surrogate species could also be used for tissue samples. Such samples could be limited to muscle or organ tissues, or a whole-body sample could be taken. Appropriate sample procedures for accumulation analyses are given in Southworth and Peterson (1993) and EPA (1993c).

Methods for Chemical Analysis

No widely accepted or official methods exist for chemical analyses of biological tissues. The EPA has developed two different sets of analytical protocols for water and soils: SW846 (EPA 1986) and the Contract Laboratory Program (CLP). Normally CLP procedures must be used for analyses of contaminants at CERCLA sites. However, there are no CLP procedures for biological tissues. Because biological tissues differ physically and chemically from water and soil, neither the SW846 procedures nor the CLP protocols are directly applicable. Although most of the steps in either set of protocols may be applicable to biological tissues, some changes need to be made.

This section provides general information that may be useful in developing the analytical portion of a sampling and analysis plan. In virtually all cases, the best

way to ensure that analytical data are of sufficient quality is to follow two guidelines: (1) to the maximum extent possible rely on existing EPA-approved procedures, varying only in those steps where biological tissues differ from the sample matrix for which the procedure was developed, and (2) always use analytical laboratories that have performed analyses of biological tissues in the past and that are part of an ongoing performance evaluation program based on biological tissues.

Four key areas in which decisions must be made about the best approach for analysis of biological tissues are: (1) sample weight, (2) sample preparation prior to shipment, (3) sample preparation in the analytical laboratory, and (4) deviations from standard analytical protocols. These decisions should be made in conjunction with a skilled analytical chemist familiar with the methods that are available for the sample of concern. Where possible, the laboratory that will perform the analyses should participate in these decisions.

Sample Weight

The weight of the sample available to the analytical laboratory affects the sensitivity of the analysis and the level of quality control that can be implemented. All laboratories establish certain minimum weights that can be analyzed to provide specified limits of detection or quantitation. In general, the more sample available, the lower the limit of detection until the method detection limit is reached. Larger sample weights also provide sufficient material for laboratory duplicates and spikes as appropriate.

Sample Preparation in the Field or Close Support Laboratory

Most biological samples are simply frozen in the field and shipped directly to the analytical laboratory. In some cases, samples may be freeze dried; however, freeze drying will volatilize some contaminants.

Recently, some ecological risk assessors and regulators have begun considering whether samples should be washed before freezing. The concern is over whether contamination on the outside of the sample is likely to be biologically available, either to the organism being sampled or to its consumers. If the question to be answered is whether internal contamination within an organism (e.g., a surrogate for a T&E species) is sufficiently high to indicate adverse effects, then washing the exterior of the sample removes the possibility that spurious external contamination is influencing the results. On the other hand, where external contamination is potentially part of the exposure pathway (e.g., in plants consumed by an herbivore), the exterior of the sample should not be washed. Of course, if a sample is washed,

samples of the detergent should be analyzed to determine if it is adding any contamination.

Holding times have been established for most analytes in soil or water, but there is no consensus on holding times for biological tissues. If tissues are frozen shortly after collection (and washing if required), then contaminants bound up in the biological tissues should remain there for a relatively long time. However, where concentrations are to be considered against ARARs (applicable or relevant and appropriate requirements) that are based on wet weights (e.g., Food and Drug Administration [FDA] threshold limits), lengthy time in a freezer can result in water loss that will affect measurements of wet/dry weight ratios. In general, the best practice is to ship samples to the analytical laboratory as soon after collection/preparation as possible.

Sample Preparation by the Analytical Laboratory

Standard analytical procedures typically are divided into (1) the preparation steps necessary to get the sample into a form that can be analyzed and (2) the actual analytical steps. Preparation steps for biological tissues depend entirely on the type of tissue (e.g., hair, organ, whole body, or plant tissue) and the analyte.

In general, analyses of all tissues for trace metals requires total dissolution of the sample. Such preparation may include a step in which the sample is physically altered by grinding or other means, followed by various chemical treatment steps, usually involving various strong acids. The end product is a clear liquid that can be analyzed by the appropriate process.

Analyses for organic compounds vary considerably depending on the class of compounds. In most cases, the preparation step involves extraction of the organic compound from the sample by some method. The extracted solution must be cleaned to remove lipids and compounds that may interfere with the analysis. Detailed discussion of all organic analytical methods is beyond the scope of this document. Refer to the standard analytical methods for the compounds of interest.

Deviations From Standard Analytical Procedures

Once a sample is prepared for instrumental analysis, the differences between biological samples and other matrices are largely gone. Deviations in this area generally relate to different quality control (QC) steps. For example, there may not be an adequate blank sample for a biological tissue, so deionized water may be used. Spiking procedures and expected recoveries may vary from those stated in standard

methods. It is important, however, that the basic QC requirements of the standard protocols be followed. There is no need to eliminate QC steps such as blanks, internal lab duplicates, or standard reference materials just because the sample matrix is a biological tissue.

7 Biological Survey Methods

Biological survey data reflect the actual health of biological populations and communities in the field and are indicators of the presence or absence of toxic ecological effects. For example, if the ecological parameters (abundance and productivity of endpoint species, species diversity, etc.) measured at a smoke-exposed site are statistically similar to those observed at reference locations, the conclusion that smokes do not adversely affect these ecological parameters measured is supported. In contrast, significant differences between the exposed and reference sites suggest contaminant effects; toxicity test data and conventional toxicity data are needed to verify this conclusion.

Biological surveys may provide evidence of both direct and indirect effects of smokes and obscurants on T&E species. To evaluate the likelihood of indirect effects, condition and availability of habitat and food required by the T&E species must be measured, in addition to the abundance and productivity of the endpoint species itself. Methods that may be used to collect these data are summarized in the following sections. Although some methods are described in reference to a particular taxon, with slight modification they may also be applied to other taxa.

Plants

Monitoring Abundance and Distribution of T&E Plants

Threatened and endangered plant species have specific habitat requirements that will aid in field investigations to locate these species. These requirements may include soil type and drainage, degree of slope, direction of slope, degree of shading, elevation, or presence and density of other plants. In rare cases, a specific pollinator may be required. Contacts within the USFWS or appropriate state agencies can provide specific information on habitat requirements. Literature sources such as the World Wildlife Fund's (WWF's) endangered species guides (Lowe, Matthews, and Moseley 1994) also provide general information on habitat requirements.

Once suitable habitat has been identified, a competent field botanist who is familiar with the plant species should survey the area. Although a thorough inventory of the

suitable habitat is desirable, it may be necessary to use transects for low-growing or cryptic plants or where the size of the area precludes a complete inventory.

The botanist should mark the location of any individual plants found and note whether the plants are showing any obvious signs of stress. Locations of specific T&E plants can be mapped using standard surveying techniques or with portable global positioning system (GPS) units (provided a means of correcting for dithering error is used). This map can be used to guide decisions about changes in locations where smokes and obscurants are used. Also, over time, repeated field surveys and mapping will provide a picture of how the population is responding to exposure or remedial actions.

Monitoring To Identify Habitat Suitable for T&E Wildlife Species

Plants provide the most important component of habitat requirements for T&E wildlife species. Most T&E wildlife species are threatened or endangered primarily because of loss of critical habitat. Identifying the presence of suitable habitat (over a large enough area) is the first step in determining whether a given T&E species is likely to be present. Also, for many T&E wildlife species, the USFWS or appropriate state agency considers preservation of critical habitat analogous to protection of the species.

Three basic habitat variables can be directly measured and used to predict habitat suitability: foliage density, species composition, and fruit production (Anderson and Ohmart 1986). Of these, species composition is perhaps most useful for many rodent and bird species (Anderson and Ohmart 1986). A number of other variables can be derived from these basic measurements, but these indirect variables may be less helpful in locating habitat for specific wildlife species.

Foliage density is the amount of foliage per unit area or to the extent of canopy cover. Plant density (the number of plants per unit area) is not the same as foliage density. Foliage density generally is measured at different vertical levels within the vegetation. Canopy-cover requirements for species may be related to types of vegetation such as herbs, shrubs (defined either by height or diameter limits), or overstory. Habitat requirements for some species (e.g., cavity nesters) may include a minimum number of snags (dead trees) or downed logs per area. Other species (e.g., small mammals, amphibians, and invertebrates) may require a degree of forest floor litter cover.

Foliage density can most easily be measured using a transect system. Transects are established either randomly or in representative areas. At predetermined points

along each transect (e.g., every 5 m), the canopy cover or foliage density is measured at each desired vertical level. Quadrat methods may be used alone or in conjunction with transects. Quadrats are predetermined areas (frequently 1 by 1 m squares or 1-m-diameter circles) that are sampled to estimate the foliage density or canopy cover. Transect and quadrat methods are best suited for use with low-growing species or large areas. Further details on these methods are in Hays, Summers, and Sietz (1981), Anderson and Ohmart (1986), and Higgins et al. (1994).

Fruit production refers to the quantity of fruit produced by plants. These items can be attractive to some T&E wildlife species of interest. Mast surveys conducted by many state wildlife agencies are an example of this parameter. For many species of plants, fruit production does not correlate well with the number of individual plants present (Anderson and Ohmart 1986). In these cases, it may be necessary to measure fruit production for a representative number of individual plants.

For certain species (e.g., some birds) the degree of patchiness or the amount of edge habitat per unit area may be important. These parameters are best measured from large-scale vegetation cover maps derived from aerial or satellite data. The use of computerized geographic information system (GIS) procedures can greatly enhance analysis of habitat patchiness.

Birds

Biological survey methods for birds include four categories: population survey, nest study, food habit survey, and habitat evaluation. Habitat evaluation methods focus primarily on measurements of plant distribution and structure of the vegetation community and are discussed in the "**Plants**" section of this chapter. Representative methods for the remaining categories are described in the following.

Avian Population Survey Methods

Many methods are available to determine the abundance, density, and spatial distribution of birds. These methods may be used to census populations of a single species (i.e., the T&E species of interest) or to census the entire avian community in a given area. The commonly used methods include territory mapping, transects, point counts, mark-recapture, song-tapes, aerial counts, and habitat-focused surveys.

Territory mapping. Territory mapping is among the most accurate and reliable methods for determining bird population density (Wakely 1987a). This method consists of using a sampling grid to plot (by individual species) the locations of birds

seen or heard during eight to ten repeat visits (Verner 1985; Ryder 1986; Wakely 1987a). Clusters of observations are assumed to represent the center of activity for individual territories. The total number of birds on a plot is then estimated by summing the number of clusters (e.g., territories) and multiplying by two (assuming an even sex ratio) (Verner 1985). This method works best for species that sing conspicuously from within their territories (e.g., most passerines). It is not well suited for birds that frequently sing within the boundaries of a conspecific's territory, quiet or secretive species, nonterritorial birds (e.g., floaters), or species with territories larger than the study plot (Verner 1985). Also, because the efficacy of this method depends on territorial behavior, it is useful only during the breeding season (except for birds that maintain year-round territories). This method also requires considerable time to lay out and mark the sampling plot and for repeated visits. Additional limitations of territory mapping are summarized by Oelke (1981).

Falls (1981) reports that detection of individuals may be enhanced by using playback of recorded songs. Birds defend their territories in response to the recorded song and their singing locations indicate a territory's boundary.

The consecutive-flush technique (Whitmore 1982; Verner 1985) may be used to reduce the number of plot visits needed to complete a territory map. An observer simply approaches a singing bird until it flushes. Its initial position, line of flight, and landing position are then recorded on the plot map. The observer again approaches and flushes the bird and records its movement. The process is repeated until at least 20 consecutive flushes have been mapped. This technique is most applicable in open habitats such as grasslands or marshes, where an observer may keep a individual bird under constant observation. Flushing may also help delineate territory boundaries in forested habitats (Verner 1985).

Transects. Transect census methods consist of counting birds either seen or heard along one or both sides of a line through one or more habitats (Ryder 1986). Transects are more flexible than are mapping methods. Because they do not depend on territoriality, their use is not restricted to the breeding season. In addition, they may detect both floaters and juveniles. Verner (1985) defines three general types of transects:

1. Line transects without distance estimates. The observer simply walks a preset line and records all birds seen or heard, without measuring or estimating distances to the birds. This is an efficient method for generating lists of species. However, the results cannot be used to estimate density because the area sampled is unknown. Data may be used for intraspecies or interspecies comparisons (either temporal or spatial), if it is assumed that all

individuals or species are equally detectable in all samples and factors that affect detectability are similar among all samples.

2. Variable-width line transects. This is the most commonly used transect method. Perpendicular distances from the transect line to birds detected are measured or estimated. These observations are then used to estimate the area sampled and, thus, bird density.

3. Belt transects. This method is essentially a line transect with fixed boundaries (usually 25 to 50 m on either side of the line), within which all birds seen or heard are counted. This is a simpler method than the variable-width transect method because the observer need only estimate one distance, the belt width. Density estimates are obtained by dividing the total number of birds observed by the area of the belt.

Burnham, Anderson, and Laake (1980) provide a very detailed discussion of line-transect techniques, applications, and data analysis methods. Additional discussion is provided in Wakely (1987b). Analytical methods for line-transect data are discussed by Krebs (1989).

Point counts. Point counts consist of counting the number of birds seen or heard for a fixed time in all directions from a single point. Similar to transects, distances around the sampling point may be undefined, fixed, or variable (Verner 1985). With the variable circular plot method (Reynolds, Scott, and Nussbaum 1980), the distance from the sampling point to the bird is estimated. This distance is then used to estimate the population density. Because point counts do not depend on territorial behavior, they may be performed year-round. Best results, however, are obtained during the breeding season. Although point counts may be performed in any habitat where transect sampling would be applicable, they are best suited for steep, rugged, or thickly vegetated habitats where observer movement along the transect may disturb birds and interfere with their detection (Reynolds, Scott, and Nussbaum 1980; Ryder 1986; Wakely 1987c). Use of point counts to survey birds in bottomland hardwood forests is discussed by Smith et al. (1993).

Mark-recapture. The ratio of marked individuals to unmarked individuals may be used to estimate population size. Population size and area sampled can then be used to estimate density. Karr (1981) suggests using mist nets (see Chapter 6, ***Birds, Methods***) to capture and color-band birds for population studies. Although mark-recapture is not considered an efficient population census method for birds (Verner 1985; Ryder 1986), it may provide very useful information, particularly in studies of T&E species. For example, mark-recapture data may be used to identify

the number of pairs of a species that are present, to distinguish migrants from residents and breeders from nonbreeders, or to identify ranges or territorial boundaries for individual birds (Ryder 1986). Additional discussion of the use of mark-recapture to estimate avian populations is presented by Nichols et al. (1981) and Jolly (1981). Analytical methods for mark-recapture data are discussed by Krebs (1989).

Song tapes. Censusing inconspicuous or secretive birds (i.e., nocturnal, marsh, or some forest birds) may be very difficult. Johnson et al. (1981) and Marion, O'Meara, and Mohair (1981) suggest that song tapes may be used to perform relative or absolute censuses for these species. By playing recordings in different areas and recording occurrence and number of responses, presence, abundance, and density may be estimated.

Aerial counts. Large flocks of waterfowl and shorebirds may be photographed from the air and later counted (Verner 1985). Aerial counts are also suggested for breeding ospreys (Swenson 1982). Because osprey nests are large, conspicuous, and generally placed in trees or atop artificial structures, they can be clearly observed from the air. Census flights should be made during the incubation period (generally April through June) using a high-winged aircraft or a helicopter. Krebs (1989) discusses analytical methods for aerial survey data.

Habitat-focused surveys. Habitat-focused surveys are particularly suited for T&E species. First, areas with critical habitat are identified, and then the presence, abundance, and distribution of the target species is determined. By focusing on a particular, critical habitat (usually nesting habitat), the likelihood of finding the T&E species and collecting data relevant to an ERA is increased. For example, Thompson (1982) describes a habitat-focused survey method for the red-cockaded woodpecker.

Red-cockaded woodpeckers are a colonial-nesting T&E species that require mature, open, fire-maintained pine forests (Thompson 1982). Survey methods for this species rely on identification of appropriate habitat (old-growth pine forest) and nest-trees within the habitat (large-diameter trees with clear boles and flattened crowns). Habitat and trees within habitat may be identified using a combination of remote sensing and ground truthing. Presence of red-cockaded woodpeckers in an area is indicated by:

- 2-in. diameter cavities excavated in living sapwood
- chipping of small wounds (resin wells) in the pine bark
- flow of pine resin from cavity and resin wells, giving tree a glazed appearance
- flaking of loose bark from the tree cavity.

Once the presence of red-cockaded woodpeckers in an area has been verified, the population size may be determined by observing the activity at the cavities and counting the number of individuals observed (Thompson 1982).

Additional information. Much has been written on avian censusing techniques. Detailed discussions and comparisons of census methods, methods for analysis of census data, sampling designs for avian censuses, and factors that affect census results are presented in Ralph and Scott (1981). Chapters concerning census methods for songbirds, raptors, shorebirds, waterfowl, colonial waterbirds, and upland gamebirds may be found in Cooperrider, Boyd, and Stuart (1986). Davis (1982a) presents census methods specifically for 43 species of birds and 14 groups of birds or birds in specific habitats.

Avian Nest Study Methods

The nesting stage is critical for all birds. Any environmental factors that affect birds during this stage and reduce recruitment may have adverse population effects. One way to evaluate whether recruitment is being affected is to calculate nest success.

The Mayfield method is the most common way of calculating nest success (Mayfield 1975). This method considers the survival of a nest over the time that it is observed. In practice, the daily survival rate is estimated by dividing the total number of young or eggs lost by the total number of days the nest has been observed and subtracting this quotient from 1. This value represents the probability of survival for the nest during that period. By analyzing the time frame of the different nesting stages (i.e., laying, incubating, nestling, etc.), investigators can identify the stage at which mortality is occurring. Applications and mathematical validity of the Mayfield method are discussed by Miller and Johnson (1978), Johnson (1979), Hensler and Nichols (1981), and Winterstein (1992).

Nest attentiveness is another factor that may affect nest success and, thus, recruitment. Grue, Powell, and McChesney (1982) observed that European starlings exposed to a sublethal organophosphate insecticide dose fed their nestlings less frequently and were away from the nest longer. Nestlings in nests of exposed birds lost weight. Because fledging weight is correlated with survival (Perrins 1965), altered nest attentiveness may cause negative impacts to avian populations.

Methods to monitor nest attentiveness or activity include visual observations (e.g., Heagy and Best 1983), time-lapse cameras (e.g., Grundel and Dahlsten 1991), telemetric eggs (e.g., Varney and Ellis 1974), and radio-equipped birds (e.g., Licht et al.

1989). Additional methods for cavity nesting birds are discussed by Mallory and Weatherhead (1992).

Avian Food Habit Study Methods

Food habit studies have two primary applications in risk assessment. First, they may be used to identify and quantify contaminant exposure pathways through the food web. Samples of food consumed, excreta, or rejecta may be collected and analyzed for residues and to determine diet composition. Second, use-availability studies or foraging behavior studies may be performed to evaluate if indirect effects are occurring that may affect the energetic status of the species in question.

Methods for performing avian diet analysis have been reviewed by Rosenberg and Cooper (1990) and are summarized in Table 3. Data may be presented as percentage occurrence (number of samples in which a food item appears), frequency (number of times a food item appears in a sample), or percentage volume or weight (proportion of total sample volume or weight accounted for by a food item). To prevent confusion and minimize bias, both frequency and volume data should be reported. For example, an important food type may be consumed in high volume but low frequency. Conversely, a food of minimal importance that is highly abundant may be observed in high frequency but low volume.

For additional discussion of methods and approaches to investigating avian eating habits, consult Morrison et al. (1990). This volume includes papers that discuss approaches to quantifying diets, design and analysis of foraging behavior studies, use-availability analysis, energetics, and foraging theory. Additional methods for analysis of use-availability data, niche overlap, and dietary data are described in Krebs (1989).

Mammals

Most T&E mammal species are nocturnal (e.g., most carnivores) and/or cryptic (e.g., shrews). Thus, unlike some other taxa (e.g., birds), direct observation and counting are feasible only for a few mammal species. In addition, census methods for most mammals are notoriously inaccurate and mammal populations tend to fluctuate greatly. For most mammals, therefore, measurement of demographic effects (e.g., changes in population sizes) is unlikely to provide valid comparisons between contaminated and reference sites. For these reasons and because the unit of assessment for T&E species is the individual rather than the population (see Chapter 2), this section focuses primarily on methods for determining the presence

and distribution of mammals. Some of these methods, if used in a rigorously designed approach, may also be suitable for estimating population sizes.

Three general approaches to biological surveys of mammals have been widely used: habitat suitability surveys, direct measurements (e.g., observation of the animal or trapping), and indirect methods (e.g., scat surveys or scent stations). Because direct and indirect measurements are both time-consuming and expensive, the best strategy is to first determine whether suitable habitat exists in the target area. If suitable habitat does exist, then either direct or indirect methods may be used to

Table 3. Comparison of common methods used to obtain avian diet samples.

Method	Advantages	Disadvantages	Example of Use
Direct examination of collected birds	Whole stomachs collected; if shot, then exact bird desired can be obtained.	Birds are killed; multiple samples from one bird not possible.	Rotenberry (1980) Sherry (1984)
Chemical emetics	Birds not directly killed.	Mortality may still be substantial; multiple samples from one bird often results in mortality; birds must be captured; partial samples obtained; unsuitable for some species	Zach and Falls (1976), Robinson and Holmes (1982), Gavett and Wakely (1986)
Stomach pumping	Birds not killed	Birds must be captured; partial samples obtained	Moody (1970)
Fecal samples	Birds disturbed minimally; samples easily obtained.	Birds usually must be captured; samples highly fragmented; samples must be treated before analysis.	Ralph et al. (1985)
Ligatures	Arthropod prey usually intact; can be effective when combined with direct observation.	Restricted to nestlings; feeding behavior and survival can be affected; estimates of prey size can be biased.	Johnson et al. (1980)
Pellets	Birds not disturbed; samples easily obtained; keys to mammal skulls and hair available.	Restricted to pellet forming species; may be biased by prey type, size.	Errington (1930)
Direct observation (adult birds)	Birds not disturbed; foraging behaviors that result in prey captures are observed.	Difficult for insectivorous birds; observations biased towards large conspicuous prey.	Robinson and Holmes (1982), Price (1987)
Direct observation (nestlings)	Birds not disturbed; can be effective when used in conjunction with ligatures.	Time consuming; labor intensive; biased as above.	Johnson et al. (1980)
Photography	Birds not disturbed; automatic movie cameras provide many samples for little effort.	Restricted to nestlings; equipment relatively expensive; hand-operated cameras time consuming, labor intensive.	Royama (1959, 1970), Dahlsten and Copper (1979)

Source: Rosenberg and Cooper (1990).

determine if the T&E mammal is present and to estimate the species distribution and, possibly, abundance.

Habitat Suitability Measurements

As discussed in the plant portion of this chapter, animal habitats are governed largely by the taxonomic composition and structure of the plant community. Habitat information for T&E species is available from a number of sources. The USFWS has developed a habitat evaluation program and a number of habitat suitability models for individual species. USFWS contacts for many endangered species are listed in the WWF's three-volume set on endangered species (Lowe, Matthews, and Moseley 1994). Each of these USFWS contacts should be able to identify key habitat requirements for the species of concern. Lowe, Matthews, and Moseley (1994) also presents a summary of habitat requirements and a brief bibliography for each species covered. For those species not covered, search the open literature and make contacts with regional USFWS offices and appropriate state wildlife agencies.

Once habitat requirements for a given T&E species are known, the area likely to be impacted by smokes and obscurants should be surveyed to determine if suitable habitat is present. Habitat survey methods related to plants were reviewed briefly earlier in this chapter. Other survey techniques are included in Cooperrider, Boyd, and Stuart (1986); Hays, Summers, and Sietz (1981); and Higgins et al. (1994).

GISs should be considered to facilitate the comparison of areas containing suitable habitat for T&E species and smoke-impacted areas. Koeln, Cowardin, and Strong (1994) review the application of GIS technology to wildlife habitat evaluation.

Direct Methods of Surveying Mammals

Direct observation of nonflying mammals. Direct observation is rarely a reliable method for surveying mammals, especially in forested habitats. However, in open terrain, some large mammals can be censused by aerial surveys. This is especially effective for large ungulates such as elk and bighorn sheep (Davis 1982a) and wolves (Fuller 1982). Other direct observation methods are described in Davis (1982a).

Another technique that is used frequently with predators is vocalization surveys, which may be of two types. In the first type, a recording of the animal of concern is played and the number and direction of responses are noted. In the second type, a recording of a wounded prey animal is played, which may elicit movement of the predator into observation range.

Trapping of nonflying mammals. Where direct observation is not possible, many mammal populations may be surveyed by trapping. Trapping of T&E species requires special permits from the USFWS (for Federally listed species) or the appropriate state (for state-listed species). A critical concern in trapping T&E species is that virtually all trapping systems involve some risk of injury or death to the captured animal. Because the unit of risk assessment for T&E species is the individual, trap-related death or injury is a serious concern. Sampling plans and permit applications must include steps to mitigate or minimize this risk. USFWS permitting procedures require a 30-day public comment period and resolution of comments. Therefore, permit applications must be submitted months in advance of anticipated field work.

Trapping of some mammal species was described in Chapter 6. Additional trapping techniques are discussed by Davis (1982a), Cooperrider, Boyd, and Stuart (1986), and Schemnitz (1994). Health concerns related to disease transmission are significant when handling wild animals. Rabies, hanta virus, tetanus, and various parasites may be transferred to humans, sometimes with fatal results. See Chapter 6, ***Mammals*** for a discussion of precautions related to these health concerns.

If population or distribution information is desired, trapping the animal is only the first step. Various techniques have been used for population estimation by means of repeated captures of marked and unmarked animals (Davis 1982b). Home range information can be obtained by radiotelemetry (Samuel and Fuller 1994; Davis 1982a), but telemetry is most effective for larger animals (i.e., minks or larger).

Indirect Methods for Nonflying Mammals

For T&E species, indirect methods offer the advantage that they do not involve harassment or possible harm to the animal. They also do not require issuance of USFWS or state permits. Probably the two most widely used indirect methods of surveying for the presence and distribution of animals are scat (fecal material) surveys and scent stations (Morrison et al. 1981). Both methods can provide evidence of the presence of an animal at a specific location.

Scat surveys are generally accomplished by laying out a series of transects through the area of interest and collecting scats from those transects. Various resources exist to facilitate the identification of scats (e.g., Murie 1974). Scat surveys are also useful in determining the dietary composition of the animals of interest.

Scent stations are generally small (e.g., 1-m-diameter) areas, cleared and prepared with a smooth tracking surface (e.g., powdered lime, sand, or sifted dirt). A scent

attractive to the species of interest is placed near the middle of the prepared surface. Scent stations are laid out on a transect along travel routes used by the animals of interest. When the station is unattended (typically overnight), animals visiting the scent leave their tracks in the surface. When the researcher next visits the station, the tracks can be identified. This technique relies on the tracking skill of the researcher and favorable weather conditions, since rain or wind can destroy tracks and the station.

Surveys of Bats

Bats are one of the few orders of mammals that can be adequately surveyed via direct methods. In fact, indirect methods are much less effective than direct methods for bats. Survey and census methods for bats are reviewed by Thomas and LaVal (1988). Kunz and Kurda (1988) describe bat collection techniques. Of course, the permitting and injury minimization issues discussed earlier in this chapter apply equally to bats. Health precautions for bat researchers are discussed by Constantine (1988). Fenton (1988) discusses the use of vocalization recording to identify and survey for bats. Techniques for reproductive assessment, food habits analysis, and other ecological monitoring are described in Kunz (1988b).

Reptiles and Amphibians

Habitat Evaluation

Habitat and microhabitats can play a critical role in the presence or absence of reptiles and amphibians. Jones (1986) considers horizontal and vertical vegetation habitat availability to be the most important factors affecting reptile and amphibian distribution and habitat use. Habitat availability is considered more important than food abundance in species distribution for terrestrial reptiles; the inverse is true for aquatic and subaquatic species (Reynolds and Scott 1982). For example, certain basking turtles can be eliminated from ponds if floating logs are removed (Jones 1986). Soil type is an important limiting factor for tortoises (Luckenbach 1982); the soil must be loose enough for digging but firm enough so that burrows will not collapse. Amphibians are restricted to areas that contain suitably moist or wet habitats. Free-standing water may be required for larval frogs, toads, and salamanders to develop to the adult stages. Many salamanders require moist rotting logs or leaf litter, both for egg development and adult survival (Stebbins 1966).

To determine the presence, quantity, and quality of critical habitat for T&E reptile and amphibian species at a smoke-exposed site, a habitat survey must be

performed. A description of critical habitat should include the general type of aquatic and/or terrestrial habitat (e.g., large river, spring, swamp, pond, forest, grassland, or desert) and proceed through the microhabitat. The microhabitat description should focus on habitat required for breeding (e.g., egg, larval, or juvenile habitat), adult survival, and needs and food requirements of each life stage. The assessment of microhabitats should include a determination of accessibility between these areas and other microhabitats required by the T&E species and possible barriers between microhabitats. Survey methods for aquatic habitats are outlined in Chapter 7, ***Fish, Habitat Assessment***. Similar techniques generally can be used to evaluate habitat for aquatic reptiles and amphibians.

Surveys of terrestrial habitats may include a description of the general habitat type (e.g., grassland or upland forest), soil composition, slope aspect, vegetative cover, leaf litter, air temperature, humidity, and rainfall. Many texts, papers, and manuals contain detailed descriptions of methods for vegetation sampling and analysis and habitat evaluation (e.g., Mueller-Dombis and Ellenberg 1974; Hays, Summers, and Sietz 1981; Greig-Smith 1983; EPA 1989; and Bookhout 1994). Vogt and Hine (1982) describe a simple method for herpetofaunal habitat assessment. Beiswenger (1988) provides techniques on amphibian habitat description and modeling. Additional information pertaining to terrestrial habitat survey methods are presented in Chapter 7, ***Plants, Monitoring To Identify Habitat Suitable for T&E Species***.

Sampling Techniques

Methods selected to sample reptiles and amphibians will vary depending on the type of habitat, time of year, weather conditions, and age of target species. Although some techniques are more effective than others, careful consideration must be given to the rates of injury and mortality associated with the various methods, especially when dealing with T&E species. Some methods are lethal to many or most individuals being collected (e.g., shooting, baited hook, and chemicals). These methods are not suitable for sampling T&E species, but may be used to collect non-T&E species for residue analysis. Representative techniques for censusing and sampling reptiles and amphibians in aquatic and terrestrial habitats are listed in the following sections. Additional methods for amphibians (many also applicable to reptiles) may be found in Heyer et al. (1994).

Seining. The use of small-mesh seines (7 mm or smaller) is moderately effective for sampling of aquatic salamanders, frogs, snakes, and turtles (Jones 1986). This method generally requires at least two people to operate the seine. Other personnel

are beneficial for disturbing the substrate, blocking potential escape routes, and handling the catch.

Gigging. Gigs may be very effective for sampling frogs but are less effective for aquatic snakes. Gigging is usually done at night with headlamps. It may be done alone, but safety considerations make this inadvisable. A disadvantage of this method is that it sacrifices the organism.

Electrofishing. Although developed for sampling fish, electrofishing may also be very effective for aquatic salamanders and aquatic snakes (Jones 1986). This method occasionally yields turtles, sirens, and hellbenders. Electrofishing requires two or more people (a shocker and a netter) and is most effective in shallow water (streams, ponds, and shallow rivers). Deep-water habitats (lakes, reservoirs, and embayments) may be shocked from boats, but this approach is probably less effective for most herpetofauna than for fish. One disadvantage to electroshocking is that it may cause some mortality, especially in hot weather. See the fish sampling methods portion of this chapter for further details.

Nets and traps. Numerous types of nets and traps are available for sampling herpetofauna. Traps are generally effective for alligators, turtles, and snakes. Stebbins (1966), Conant (1975), and Shine (1986) discuss various aquatic trapping methods. Some traps may be set by one person. To prevent inadvertent mortality from trapping, traps should be checked at least daily (trap mortality is generally low if checked often). Aquatic traps should be set partially above water line to permit the captured organisms to breathe. Forster (1991) reports a gill-netting technique for alligators. Haul seines and cast nets have also been used to capture alligators (Chabreck 1963).

Shooting. Firearms can be effective for sampling frogs, turtles, and alligators. Frogs and alligators may be sampled at night using headlamps or hand-held spotlights. Firearm sampling requires experienced personnel and strict adherence to safety procedures. Accurate, high-powered air rifles (for frogs only), 0.22 rimfire (if ricochets do not pose a human hazard), or shotguns (preferable for collecting alligators) may be used. Although sampling of frogs and turtles may be performed by a single investigator, this is not advisable. Disadvantages of this method include injury or mortality to specimens and metal contamination from projectiles.

Baited hook. This method is effective for turtles and alligators. Use of baited hooks is not recommended when T&E species may be present because mortality may be extremely high.

Noosing. Noosing is a live-capture technique for alligators. This method requires two or more experienced personnel. It is generally done at night from boat or airboat, using a hand-held spotlight to immobilize the animal. Mortality from noosing is presumed to be low.

Chemicals. Reptiles and amphibians may be collected using chemicals in a manner similar to that used for fish (see Chapter 7, **Fish Sampling Methods**).

Drift fences and pitfall or funnel traps. Pitfall traps consist of a cup or bucket buried to the rim in the ground. They are effective for sampling many species of reptiles and amphibians, including lizards, skinks, salamanders, small turtles, small snakes, and toads. They are not effective for large or arboreal snakes or treefrogs. Trapping success is generally expressed as individuals per trap night (TN) or per 100 TN (Vogt and Hine 1982, Mengak and Guynn 1987, Bury and Corn 1987). Funnel traps are wire enclosures with funnel-shaped entrances through which reptiles and amphibians may enter easily but have difficulty exiting. Funnel traps are deployed above ground and are generally used with drift fences. However, they also have been successfully deployed along logs and rock ledges (Fitch 1951). Funnel traps are more effective than pitfall traps for snakes and lizards (Vogt and Hine 1982). Construction of a drift fence array requires approximately 5 person-hours, but little maintenance is needed thereafter (Campbell and Christman 1982). Use and efficiencies of drift fences are discussed further by Gibbons and Bennett (1974), Bennett, Gibbons, and Glanville (1980), and Friend (1984).

Cover boards. Relative abundance of terrestrial salamanders may be estimated using cover boards (DeGraaf and Yamasaki 1992). Boards (1 m long x 20 cm wide x 2.5 cm thick) are laid on the ground in contact with litter along transects within forested habitat. The boards simulate fallen logs, under which many terrestrial salamanders (and other amphibians and reptiles) reside. Boards are lifted periodically and the salamanders are either counted or collected. This method is less labor intensive than pitfall trapping and does not degrade habitat by disturbing litter or breaking existing logs (DeGraaf and Yamasaki 1992).

Fixed-area plots. This is an effective herpetofaunal sampling technique that involves a thorough, manual search of a relatively small plot. Searching is continued for a fixed amount of time (Szaro et al. 1988) or until all litter and debris have been overturned and thoroughly examined (Bury and Raphael 1983). Attempting to collect all individuals in a specified plot is often referred to as quadrat collecting (Campbell and Christman 1982). A quadrat may also refer to a particular microhabitat, such as tree buttresses (Heyer and Berven 1973).

Time-constraint collecting. This technique consists of a timed search of specific (prime) habitat types for certain species without using a designated plot. Time-constraint collecting is generally efficient because it focuses on the most productive areas or areas where individuals are most likely to be found. This method cannot be used to estimate density or biomass per unit area because sampling is biased toward specific habitats (Bury and Raphael 1983). Campbell and Christman (1982) also discuss this method.

Road surveys. This quick and easy method may be used at various times of the day and year to survey reptiles and amphibians. Slow-speed searches at night and even surveys of road kills may yield valuable information concerning local herpetofaunal communities. This method has many biases and cannot be used for reliable estimates of populations. However, it can save time and effort in collecting and may yield rare and elusive species not obtained by other methods (Campbell and Christman 1982).

Opportunistic methods. Numerous useful methods are used for censusing reptiles and amphibians that are not true collecting methods or that have limited application. These methods include aerial surveys (Jennings, Percival, and Abercrombie 1987; Jennings, Percival, and Woodward 1988), radiotelemetry (Jones 1986), remote camera techniques (Spillers and Speake 1988), artificial cover techniques (Bennett, Gibbons, and Glanville 1980; Grant et al. 1992; Mitchell, Erdle, and Pagels 1993), frog call indices (Vogt and Hine 1982), examination of predator feces (Jones 1986), mark-recapture techniques (Gibbons 1969; Otis et al. 1978; Ferner 1979; White et al. 1982; and Szaro et al. 1988), and general searches such as turning over rocks, logs, and other objects.

The seasonal activities, secretive nature, patchy distributions, and specialized habitats of reptiles and amphibians make determining species occurrence and relative abundance difficult (Fitch 1982; Bury and Raphael 1983; Beiswenger 1988). Many authors recommend using a combination of sampling methods to obtain a more accurate population estimate (Bury and Raphael 1983; Friend 1984). Indices of relative abundance (Heyer and Berven 1973; Bennett, Gibbons, and Glanville 1980; Spellerberg 1982) and species diversity (Fleet, Clark, and Plapp 1972; Campbell and Christman 1982) can be derived for T&E reptiles and amphibians through proper application of sampling methods.

Terrestrial Invertebrates

Methods for sampling of terrestrial invertebrates are presented in Chapter 6 and Table 2. In addition to collecting samples for residue analysis, these methods may be used along with a statistically valid sampling design to estimate population size and density of terrestrial invertebrates (see Chapter 5, **Sampling Design**; Krebs 1989; Green 1979).

Benthic Macroinvertebrates

Several sampling techniques and devices are available to quantitatively survey the abundance and distribution of benthic macroinvertebrates. The species and habitat (i.e., deep vs shallow, lentic vs lotic, etc.) being sampled will at least partially dictate final methods and devices used. Brief descriptions of several sampling methods, including equipment requirements, manpower needs, and advantages and limitations of each technique are given below.

Quadrat Sampling

Quadrat sampling involves collecting all substrate and organisms within a frame placed on the bed of a lake or stream. This method is appropriate for sampling populations of sessile organisms in most aquatic systems. To optimize sampling effort, various sources (i.e., commercial fishermen, literature searches, qualitative sampling of suitable habitat) should be consulted to determine the location of target populations before sampling begins. The generalized method entails placing the quadrat frame on the substrate, collecting all material within the frame to a specified depth, and then transporting this material to the surface in buckets. Organisms are then separated from the substrate using nested box screens, identified, measured, and then carefully returned to the river. This method may be of limited value because certified divers are required in deep water for safety reasons. In addition, this sampling can be time consuming. Detailed descriptions of these methods are available in Miller et al. (1993), Miller and Payne (1993), and Bates and Dennis (1985).

Suction Samplers

Several studies (Mattice and Bosworth 1979; Verollet and Tachet 1978; Christie 1976; Gale and Thompson 1975) have used a suction-type sampling device for collecting benthic organisms from large rivers. Basically, the substrate and associated fauna were vacuumed from a given area of the streambed into a mesh

collection bag, which was then removed and taken to the boat or shore for processing. Although only one person is required to operate these sampling devices, safety concerns would suggest at least two divers, and as noted by Mattice and Bosworth (1979), having an additional diver to change bags and transport samples made the process more efficient. All authors reported that their devices were efficient in collecting organisms from various substrate types. Verollet and Tachet (1978) and Gale and Thompson (1975) reported difficulties in sampling large organisms such as mussels and crayfish, and as noted by Gale and Thompson (1975), this sampling method resulted in damage to some organisms, which was attributed mainly to agitation while in the mesh collection bag. This method of collection would appear to be extremely stressful to benthic organisms and therefore have limited value in sampling T&E species.

Grab Samplers

Grab samplers such as the Ekman, Petersen, Ponar, and Smith-McIntyre samplers have been widely used in freshwater benthic sampling. These devices are designed to engulf a portion of substrate and associated organisms, which is then hauled to the surface for processing. These devices are generally limited to use in soft sediments because a disadvantage of grab samplers is incomplete jaw closure, particularly in coarse substrates (EPA 1973). Isom (1978) reviews several types of grab samplers, their specifications, type of substrate each was designed for, and advantages and disadvantages associated with each type. These samplers generally require a boat equipped with a winch and at least two individuals for operation (Isom 1978). Because relatively heavy weights are required for sampler penetration into the substrate, unintentional mortality from crushing may occur with this method, potentially limiting its value for sample collection where T&E organisms are present.

Surber and Hess Samplers

Small-area sampling devices such as the Surber and Hess samplers may be used in small, shallow streams (e.g., Smith 1994; Smock et al. 1992; DeBrey and Lockwood 1990; Pontasch and Brusven 1988, 1989). Both the Hess and Surber samplers are designed for use in moving water and are basically a collection net attached to a metal frame. Samples are collected by placing the sampler in the substrate and agitating the sediment within the frame to a predetermined depth (generally about 10 cm). Benthic organisms are then swept by the current into the collection net. Detailed descriptions of the use of Surber and Hess samplers may be found in numerous publications (e.g., Platts, Megahan, and Minshall 1983; Isom 1978) and in Appendix A. These collection techniques are quick, inexpensive, and require only

one operator, although an additional person increases efficiency. Both samplers are suitable for shallow stream collection of most benthic macroinvertebrates, including oligochaetes, crustaceans, and aquatic insects; however, the current must be fairly strong to wash heavier organisms such as bivalves and snails into the net. Furthermore, the enclosed area (≤1 sq ft*) is too small to adequately sample larger mussels, and they are not designed for sampling in water deeper than the top of the sampler (Dennis 1985).

Corers

Coring devices can be used in both shallow and deep water to quantitatively sample invertebrates. Williams and Hynes (1973) and Godbout and Hynes (1982) discuss the design and use of standpipe coring devices suitable for sampling the interstitial spaces of coarse streambed material. The corer is driven into the substrate to a predetermined depth with the aid of a stake driver, a core rod is inserted into the standpipe, and the sample is removed. Corers have also been used to sample invertebrate populations in areas with fine particle substrates (Smock et al. 1992; Strommer and Smock 1989). Equipment required for core sampling benthic organisms inhabiting interstitial spaces includes an appropriate coring device as well as any associated equipment for sample processing (e.g., sieve). Because it is often necessary to force coring devices into the substrate, the possibility that this method could result in injury or mortality to some organisms must be considered.

Fish

Biological surveys to evaluate potential impacts of smoke and/or obscurant chemicals on T&E fish species should begin with a literature review to determine life history characteristics and critical habitat requirements. For T&E species in the southeast, information can be found in Smith-Vaniz (1968), Douglas (1974), Lee et al. (1980), Trautman (1981), Becker (1983), Robison and Buchanan (1988), Etnier and Starnes (1993), and Jenkins and Burkhead (1993). For some species, information is summarized in the WWF *Guide to Endangered Species of North America* (Lowe, Matthews, and Moseley 1994), including contact personnel with the USFWS or other U.S. agencies.

* 1 sq ft = 0.093 m^2.

Habitat Assessment

A description of critical habitat should include the general type of aquatic habitat (e.g., large river or spring-fed tributary) with as much detail as possible down to a microhabitat level. Critical habitat is generally considered to include areas for growth, movement, nutritional requirements, reproductive and rearing sites, and adequate cover (Sidle 1987). The assessment of habitat should also include a determination of accessibility of these areas. Aquatic systems can be much more isolated than terrestrial systems because of instream barriers.

In most cases, the literature should provide sufficient detail to direct the habitat surveys in the field. If detail is lacking for a species, it may be necessary to use that in literature on a similar species. In most cases, this surrogate species will be within the same subgenus or genus classification. Once a detailed search pattern has been identified, maps, aerial photographs, or other descriptions of the base can be used to prioritize field surveys. Field surveys will focus on identifying, quantifying, and assessing potential critical habitat. General techniques for the field surveys are outlined in the following sections.

Habitat survey techniques. The two basic types of aquatic habitat are still (lentic) and running (lotic) water systems. Key components for both habitats include size, physical characteristics, chemical characteristics, and the riparian zone.

The size of an aquatic system can be measured in several ways. The total watershed area, or the area drained by all tributaries in a system, can be a significant determinant of size. The stream order classification system rates streams based on the number of tributaries within the system. This size indicator is widely used, but potentially misleading (Hynes 1970). Other measures of size are stream length, width, depth (midstream and shore), and surface area. Stream discharge is a functional assessment of stream size (Hynes 1970). The discharge is a combination of the stream width, depth, and water velocities for a particular transect. Combinations of these measures also have been recommended (Hughes and Omernik 1983). Techniques for measuring these size variables can be found in Hynes (1970), Platts, Megahan, and Minshall (1983), and Armour, Burnham, and Platts (1983).

Physical characteristics of stream habitat greatly influence the suitability of a reach for the target species. The physical characteristics reflect both variables of the water column and stream channel or lake basin and include water velocity, temperature, bank/shore structure, substrate, cover, turbidity/sediment loading, and system accessibility. Techniques to measure these variables are discussed in

Platts, Megahan, and Minshall (1983), Orth (1983), Bain, Finn, and Booke (1985), Platts et al. (1987), Bain (1988), and Baltz (1990).

Within a stream reach, there are smaller habitat types that incorporate many of the unique physical characteristics required by a T&E fish species. These microhabitats include riffle, run, glide, pool, and pocket water (Platts, Megahan, and Minshall 1983) defined by a combination of water velocity, depth, gradient, substrate type, and cover. Riffles represent shallow areas with faster currents and form some of the most important stream areas for spawning and production of food. Pools are deeper areas with slow water velocity that usually contain high numbers of fish and often are primary rearing areas for juveniles. For many fish species, the combination of a riffle and pool habitat is a critical habitat requirement.

Chemical characteristics of the water in aquatic habitats can be important determinants of whether a physically suitable site will support any T&E fish species. Important chemical variables include dissolved oxygen, alkalinity, pH, hardness, and conductivity. Unless specified in a critical habitat description for a T&E species, a value within the normal range for any of these parameters should be sufficient.

The vegetative area surrounding the aquatic habitat can greatly influence both physical and chemical characteristics of the system. This area, the riparian zone, also acts as a buffer for potential impacts from human activities (e.g., farming and logging) that increase sediment runoff or chemical inputs. Important variables for the quality of the riparian zone include sufficient width, amount and types of vegetation, land use, and amount of canopy or shading (Platts et al. 1987).

An obvious requirement for any habitat to be suitable for the T&E species is that the sites be accessible to the rest of the system. If a T&E species cannot swim to the site because of physical (e.g., dams) or chemical (e.g., presence of a toxicant plume) barriers, it does not matter how suitable all the other parameters of that site are. Many factors can act as barriers, including those that may be intermittent or temporal.

Habitat assessment indices or models. The evaluation of the habitat and especially the determination of whether sufficient critical habitat exists on a particular military installation can be facilitated by the use of habitat assessment indices or models. Habitat assessment indices provide a standard framework so that all assessments are comparable among sites and, if necessary, between installations. Many indices have been proposed (see Table 4 for some examples) for stream systems and a few for lake or reservoir systems. A widely published

approach is the use of the Habitat Suitability Index, which incorporates a detailed assessment of physical, chemical, riparian, and land-use characteristics into a model system (Terrell et al. 1982). The index values are combined with computer models that use known or estimated life-history characteristics for individual fish species to provide a predicted impact assessment for that species. The model is developed for both lentic and lotic aquatic systems.

Fish Sampling Methods

In addition to characterizing and quantifying the critical habitat at a site, the population dynamics of the target species must be measured. A wide range of variables can be measured to gauge impacts at the population level, and numerous

Table 4. Examples of habitat indices or models used to assess habitat quality of streams.

Model/Index	Parameters*		Reference
Habitat Quality Index (HQI)	late summer flow annual flow variation stream velocity cover width	streambank stability fish food abundance fish food diversity nitrate concentration maximum summer water temperature	Binns 1977
Qualitative Habitat Evaluation Index (QHEI)	substrate type substrate quality instream cover type instream cover amount channel sinuosity channel development channelization channel stability riparian width	floodplain quality bank erosion maximum depth current available pool morphology riffle/run depth riffle substrate stability riffle embeddedness gradient	Rankin 1989
Habitat Suitability Index Model	Many variables, including: percent cover substrate type percent pools average discharge average current velocity temperature (adult)	temperature (fry) temperature (juvenile) turbidity dissolved oxygen maximum stream depth average stream width	Terrell et al. 1982
Riparian, Channel, and Environmental Inventory	land use riparian zone width riparian zone completeness riparian zone vegetation retention devices channel structure	channel sediments stream-bank structure bank undercutting substrate riffles pools	Petersen 1992

*For some models, not all parameters are listed.

sampling techniques can be applied to collect the appropriate data. The following sections briefly review the applicable techniques.

Seines and mobile nets. Small seines are perhaps the most common and one of the least expensive types of gear for sampling fish (Etnier and Starnes 1993). Basically, a seine is a fine-mesh net (of varying length and height) fastened to two end poles (brailes). Floats are normally attached to the top line and metal weights or lead attached to the bottom line. The seine can be pulled through still or slow current habitats or held in place in faster currents. Detailed capture techniques are described in Etnier and Starnes (1993). Using a small seine technique includes these advantages: (1) low equipment cost, (2) small number of samplers required, and (3) low stress to target species. Disadvantages of a small seine technique include: (1) extensive sample effort required to cover large areas, (2) inefficient at sampling adults of large or fast swimming fish, (3) inefficient at sampling deep water habitats and areas with lots of cover (e.g., stumps), and (4) difficult to obtain good quantitative data (Lyons 1986). In general, seines are most useful for smaller aquatic habitats, including springs, headwaters, streams, shore areas in medium-size rivers, and shallow lakes or ponds. Also, seines are more effective if the target species is small, benthic-inhabiting (e.g., darters), or not a structure-associated species (e.g., midwater shiners).

To capture fish in large rivers, lakes, and reservoirs, technicians on boats deploy larger seines or nets such as beach seines, trawls, and purse seines. A beach seine is a long net (50 to 100 m) with an attached pocket or bag. Trawls are bag nets with leading wings that operate by being pulled through the water and sweeping fish into the net. They can be the most effective technique for sampling deep water habitats (Etnier and Starnes 1993). Purse seines are long nets with a cinching line on the bottom edge. They are deployed in a circle to surround fish, the bottom is cinched tight to form a bag, and the encircled fish are hauled into a boat by winch. These types of nets can capture large numbers of fish with limited personnel but can be stressful to the captured fish. More detailed discussions on purse seines and trawls are in Dahm (1980), Hayes (1983), and Lopez-Rojas, Lundberg, and Marsh (1984).

Electrofishing. Another very common sampling technique is the use of electric current to stun and capture fish. Electrofishing relies on a gasoline- or battery-powered generator to supply electric current (pulsed DC is most common) to a set of probes, which are placed in the water. The current passes from the cathode to the anode probe or plate. Fish in the vicinity of the electric field are immobilized through muscle contractions and can be captured easily by netters. The generators

can be of varying design and size, which allows the samplers to use backpack, shore, or boat placement.

The advantages of electrofishers include: (1) effectiveness in sampling juveniles and adults of most species, (2) effectiveness in sampling structurally complex habitats, (3) efficiency in capturing a large percentage of the individuals in an area, and (4) efficiency in sampling large areas in a relatively limited time. Numerous studies indicate that under proper conditions, electrofishing can be the most effective sampling technique (Jacobs and Swink 1982; Wiley and Tsai 1983; Layher and Maughan 1984). Disadvantages of electrofishers include (1) produces stress or fatality in a certain percentage of stunned individuals, (2) is less effective on benthic or deep water species, (3) requires clear water for most effective sampling, (4) is less effective in low- or high-conductivity water, and (5) poses potential safety problems because of high voltage and/or use of gasoline. Backpack, shore, electric seines (Bayley, Larimore, and Dowling 1989), and small boat units work effectively in springs, headwaters, streams, and medium-size rivers. Larger boat units are more effective in larger rivers, lakes, and reservoirs. In larger streams and rivers, electrofishing at night can also increase the number of species and individuals captured. Additional information on electrofishing is in Hartley (1980) and Reynolds (1983).

Stationary nets or traps. A wide variety of stationary nets and traps are used to sample fish populations. The two basic types are (1) nets that snag or entangle fish and (2) traps or net arrangements that provide a holding area into which fish are enticed. The most common entanglement nets are gill nets and trammel nets that use an open mesh through which fish attempt to swim. As the fish attempts to pass through, gill covers or fins become snagged on the fine filament netting. After a certain period of deployment, the nets are checked and captured fish are removed. These nets can be quite long and deep, and varied placement can cover a variety of habitats or water column depths. They are generally more effective in turbid water and areas without snags (Hubert 1983).

Advantages of these sampling nets include: (1) effective for a variety of larger fish sizes (depending on mesh size used), (2) effective for sampling deep areas not accessible by other techniques, and (3) effective for fast swimmers or schooling species. Disadvantages of the gill nets include: (1) snagged fish can be severely injured or killed even if nets are checked frequently, (2) any one gill net mesh size will sample only a limited size of fish, (3) nontarget species (e.g., shad species) can be captured at high rates with resulting increase in sampling time and total mortality, (4) fish species that are not especially mobile (e.g., sunfish) are not highly

susceptible to the technique, and (5) quantitative data may be difficult to obtain. Further details are given in Hartley (1980), Hamley (1980), and Hubert (1983).

Fish traps include fyke nets, hoop nets, trap nets, and pot gear (e.g., slat baskets and minnow traps). All of these devices work by allowing the movement of the fish to take them through a small opening into a larger holding area. The traps or nets are designed so that fish can easily find or be led to the opening from the outside, but not from the inside. Frequently bait is used to further lure fish into the trap (Culp and Glozier 1989). Some designs such as fyke nets use attached wing nets that funnel fish to the opening.

Advantages of these stationary traps include (1) range of sizes from small (minnow traps) to large (fyke nets) allows a wide range of species and life stages to be sampled, (2) fish remain alive while in the trap, so traps do not need to checked as frequently as entanglement nets, and (3) fish that seek cover (e.g., sunfish) or are benthic species (e.g., catfish) are especially susceptible to capture by this method. Disadvantages of these traps include (1) trap is not equally effective for all species, (2) changes in temperature and turbidity can affect catch rate, and (3) adjustments must be made to correct for sampling bias when used for density estimates (Jacobsen and Kushlan 1987). The larger fyke, trap, and hoop nets are most effective in reservoirs, ponds, lakes, and river backwaters. Pot gear and smaller hoop nets can be more effective in smaller streams or faster water. In both cases, traps can be combined with weirs or directional structures that channel fish into areas where the traps are deployed. Additional discussions can be found in Craig (1980) and Hubert (1983).

Visual observations. Using visual techniques to sample fish populations has become increasingly popular. Transect surveys of minimally or noncontaminated aquatic habitats can be effectively accomplished with snorkel or scuba gear. A small dipnet can be used while snorkeling to capture some species very effectively (Etnier and Starnes 1993). For more contaminated sites, remotely operated video cameras in waterproof cases have also been used to survey fish populations.

In both cases, advantages include: (1) minimal physical stress of fish, (2) no direct mortality, (3) effective for benthic (e.g., madtom) and midwater (e.g., minnow) species, and (4) additional information on habitat use can also be gathered. Disadvantages include: (1) not particularly useful for tissue sampling or assessment of length/weight data, (2) fish species that are difficult to identify are more easily misidentified, (3) cryptically colored species or species hiding in thick cover may be overlooked, (4) some species are more easily spooked by the diver and may be underestimated in counts, and (5) clear water is required for effective use.

Compared with seining, visual estimates can often find more species and more individuals, although abundance estimates may be biased toward smaller species (Goldstein 1978). These techniques can be applied to habitats of all sizes and under most flow conditions. Reviews of visual techniques are given by Helfman (1983), Hankin and Reeves (1988), and Baltz (1990).

Chemicals. Chemicals, or ichthyocides, have been used to sample fish populations for many years. The use of rotenone, sodium cyanide, and antimycin often provide the most complete sample of a fish community or population among all possible methods. Generally, these chemicals work by impairing the oxygen uptake at the gills, which makes the fish susceptible to capture or retrieval.

The advantages of chemical collection include: (1) thorough sampling of all sizes and species of fish, (2) effective in habitats not easily sampled by other techniques (e.g., fast current streams with large boulder substrates), and (3) specialized equipment is not required. Disadvantages include: (1) although some fish can recover, most chemical applications cause substantial mortality, (2) some chemicals can be hazardous to samplers, (3) diminished effectiveness at low temperatures, (4) technique can substantially affect nontarget species (e.g, invertebrates), and (5) application can be hard to control under some flow and habitat conditions and should only be attempted by experienced personnel (Etnier and Starnes 1993). In lakes and reservoirs, chemical application is usually limited to cove samples. Nets are used to isolate the cove, the chemical is dispersed by boat, and netters on shore and in boats retrieve the affected fish. In streams, chemicals are released at a point upstream of sample area, a line of netters retrieve fish below the sample area (may be supplemented by a blocknet), and if necessary the chemical is neutralized in water moving downstream. Chemical sampling can provide good data on standing crop, species richness, and density, but estimates are subject to bias resulting from escape around nets and incomplete recovery of dead fish (Davies and Shelton 1983). More detail on the use of chemicals can be found in Holden (1980), Kapetsky (1980), and Davies and Shelton (1983).

Data Analysis

A combination of analysis techniques is needed to properly assess the possible impact of smokes and obscurants on a T&E fish species. The selection of the most informative analysis will also depend on the sampling method selected, the amount of sampling effort possible, and the target species.

Occurrence. The first and foremost measure should be whether the target species actually occurs in the area of interest. Assuming habitat surveys have located

suitable habitat, sampling should be made to collect individual specimens. For some species this will be straightforward, while for others (e.g., shortnose sturgeon, *Acipenser brevirostrum*, or blue sucker, *Cycleptus elongatus*) the actual capture or sighting of a single individual will be a major accomplishment. Occurrence data can be correlated with areas of critical habitat to give some idea of population strength, but in most cases will need additional measures to assess potential impacts.

Catch (or counts) per unit effort/time. The easiest measure of abundance or population strength is to relate the occurrence of specimens to a unit of sampling effort, time, or distance. This catch per unit effort analysis is applicable to most sampling techniques, and when compared with reference data can indicate whether the target population may have been impacted by the installation's activities or other factors. Potential errors in using catch per unit effort for estimating fish density or biomass are discussed by Mahon (1980).

Estimated population size. For a more detailed evaluation of population size, a repeated collection estimate can be made. The two basic approaches are estimates from mark-recapture (Chapman 1951; Bailey 1951, 1952; Otis et al. 1978; Begon 1979) and removal (Zippin 1956, 1958; Seber and LeCren 1967; Carle and Strub 1978). In a mark-recapture, specimens are captured on one day, marked, and released to an enclosed sample area. After an interval of 1 hour to several days, a second collection is made. By relating the ratio of tagged and nontagged fish recaptured on the second sample day to the number of fish initially tagged, an estimate of population size can be made. Mark-recapture techniques can be limited to this single pair of sampling events, or if a long-term tag or mark is used, multiple recaptures can be made. Use of a more permanent tag will also provide data on movement patterns or distribution. Tags can be as simple as fin clips or more permanent such as plastic streamer tags, injectable wire tags, or subcutaneous paint injections (Wydoski and Emery 1983). Mark-recapture techniques may produce considerable capture and handling stress to the species and require temporally spaced sampling efforts.

The other principal method for estimating population size is the multiple-pass removal method. In this technique, an area is isolated by nets and repeatedly sampled on the same day. All target species are removed and segregated by pass. A minimum of two sampling passes are needed, and more passes (three or four are common) produce a better estimate (Riley and Fausch 1992). The target species are counted by pass and then a computer program generates an estimated population size based on the decline in numbers from the first pass to the last. This technique reduces the capture stress on the individual to one capture, and sampling can be

completed in 1 day per site, but it does not provide additional information on movements.

Regardless of technique, the estimated population data can provide a much better idea of the population size than with a catch-per-effort analysis. Data can be analyzed based on the sample area to provide density (fish/m^2) values, which make comparisons easier among sampling sites. In general, mark-recapture techniques provide more accurate estimates than removal techniques (Peterson and Cederholm 1984; Gatz and Loar 1988; Riley and Fausch 1992; Riley, Haedrich, and Gibson 1993). However, because the mark-recapture technique requires two sampling events (usually separated by a day or more between samples) and further requires tagging or marking of target species, impacts from the more accurate technique may not be worth the increased risk of mortality to the T&E species.

Additional population indicators. Depending on the desired level of sampling effort, additional measures of the target species can be made after capture. Individual fish can be measured for length and weight, observed for reproductive state, disease, or injury, and sampled for age. The length and weight measures can be made fairly easily and will provide useful data to calculate condition factor (an estimate of individual health), population biomass or productivity, and length frequencies (estimated age structure of the population). General techniques for length-weight measures are reviewed by Anderson and Gutreuter (1983). For some species, scale samples may be taken to aid in estimating age and life span. These measures are nondestructive to the individuals and should not substantially increase mortality if conducted properly.

In addition to measures of the individuals, extra surveys can focus on evaluating potential impacts on reproduction. Surveys of the critical habitat can be made during the reproductive season to locate nests or spawning aggregations of the target species or to determine the number of gravid females. Nursery areas can be sampled for the presence of eggs, larvae, or juveniles (Bagenal and Nellen 1980, Snyder 1983). These parameters can be combined with population estimates and length frequencies to indicate whether smokes and obscurants may be affecting the reproductive capacity of the target species.

8 Ambient Media Toxicity Test Methods

Toxicity tests are a means of determining if the media present at a site are actually toxic to terrestrial and aquatic biota. Testing validates toxicity estimates obtained through the comparison of contaminant exposure estimates to conventional toxicity data. The tests also indicate if differences between the smoke-exposed site and reference locations observed in the biological surveys can be attributed to contamination. This chapter summarizes representative toxicity test methods for terrestrial and aquatic biota, their applications, and their strengths and weaknesses.

Toxicity Tests for Terrestrial Biota

A variety of toxicity test methods, using organisms as diverse as earthworms, arthropods, soil bacteria, and plants, have been developed for use in assessing ecological risks to terrestrial biota at hazardous waste sites. Many of these may also be applicable to evaluate risks that smokes present to T&E species. A list of advantages and limitations of toxicity tests in ecological assessments is provided in EPA (1989).

An excellent compendium of potentially useful toxicity testing methods is provided in the *Evaluation of Terrestrial Indicators for use in Ecological Assessments at Hazardous Waste Sites* (EPA 1992b). Four main areas are covered:

1. Animal test methods (e.g., methods using earthworms, other soil annelids, free-living nematodes, soil insects, noninsect arthropods, mollusks, amphibians, small mammals, and birds)
2. Plant test methods (e.g., seed germination and root elongation tests, seedling survival and vegetative vigor tests, life-cycle and plant tissue-culture tests, and photosynthesis inhibition tests)
3. Soil biota test methods (emphasis on soil microbes, bacteria, fungi, protozoans, and nematodes)
4. Field test methods (including *in situ* testing based on organisms such as amphibians, starlings, sago pondweed, and terrestrial plants).

The American Society for Testing and Materials (ASTM) also has established guides that can be used for estimating the acute or chronic toxicity of media such as soils, sediments, and water to various organisms (ASTM 1994). The ASTM guides for these tests generally provide a greater level of detail than is provided in EPA (1992b). Each ASTM guide also contains references to the scientific literature for examples of studies that have used the method or a variant thereof.

Three key considerations were identified in the context of terrestrial toxicity testing in relation to the potential ecological risk of contaminants to T&E species:

1. The ecological attributes that cause or contribute to a species' rareness should be taken into consideration in selecting toxicity test methods. A test with an endpoint that may be sufficiently protective for an abundant species may not accurately estimate risk to a species that is rare. Toxicity tests for estimating risk to a T&E plant species, for example, almost certainly should address all critical life-cycle stages (e.g., seed germination, root and shoot development, seed set, and second-generation viability) because reproduction is generally more sensitive, as a toxicity test endpoint, than survival or growth. However, few test methods consider reproductive endpoints. Thus, the application of commonly used toxicity tests to situations involving rarer organisms may substantially increase uncertainty about potential hazards of contaminants to T&E species.

2. Although various types of organisms are used in toxicity testing, the fundamental physiological ecology or life-cycle patterns of some types of rare organisms differs so greatly from organisms commonly used in toxicity tests that the value of laboratory testing using a surrogate for the rare species is questionable. An example of a rarely considered cactus is given below to support this argument.

   Collared peccaries can discriminate among individual pads of the cactus *Opuntia phaeacantha* and preferentially eat those that contain lower concentrations of calcium oxalate (Theimer and Bateman 1992). Thus, the level of herbivory that the wild pigs exert on *O. phaeacantha* is linked to the physiological status of individual pads on individual cactus plants. Theimer and Bateman (1992) show that relatively fine-scale physiological differences can importantly influence the probability of herbivory, which is a critical source of mortality to *Opuntia*. We do not know if military smokes alter *Opuntia*'s production or storage of oxalic acid. Additionally, many species of cactus are physiologically quite different from plants commonly used in toxicity tests. Cactus species, for example, have thick, waxy cuticles and use

Crassulacean acid metabolism (CAM). In CAM plants, carbon dioxide (CO_2) is assimilated primarily in darkness and photoconverted to photosynthate in daytime, when the stomata may remain closed to protect the plant against water loss (Harper 1977). In short, CAM and non-CAM plants operate so differently with respect to CO_2 uptake, transpiration, carbon fixation, and stomatal behavior, that noncactus species of plants probably cannot be used as reasonable "surrogates" for toxicity tests designed to protect T&E cactus species. Finally, the life-cycles of some plants include very specific "ecological bottlenecks" where sensitivity to contaminants cannot be reliably estimated by using surrogate species. The pattern of seed germination and seedling survival for some species of cactus, for example, is dominated by the distribution and success of particular species of nurse plants (Valiente and Ezxurra 1991). In such cases, the cactus species would be unlikely to persist if its nurse-plant species were accidently eliminated.

3. Biological interactions such as predation, pollination, and competition may be more influential, and thus need to be considered more carefully, for rarer species than for more common species. Most commonly used laboratory or field toxicity test procedures, though, focus on direct effects of contaminants on the organism in question. Additionally, most commonly used toxicity test methods use test time scales that are too short to detect significant effects that might result from indirect mechanisms. Mesocosm-type test systems that include multiple endpoints and that continue long enough to reveal taxonomic changes in the soil community (cf. Gunderson et al. 1994; Parmelee et al. 1993) or *in situ* test systems that allow quantification of direct plus indirect effects of contaminants on community composition (e.g., Napolitano et al. 1993) require more effort than conventional laboratory toxicity tests but may provide information far more valuable in terms of assessing ecological risk of chemicals or chemical residues to T&E species.

The three considerations listed above suggest that while some laboratory- or field-based assessments of toxicity may be appropriate for estimating ecological risks resulting from exposure to training chemicals, considerable care and ecological understanding should be exercised in the extrapolation from the results of such tests, particularly those that use surrogate species, to the T&E species in question. Additionally, the considerations suggest that tests should include endpoints more sensitive than survival or growth (the most commonly used endpoints). Finally, toxicity tests that examine the effects of the suspect chemicals on specific ecological processes of significance to the T&E species in question (e.g., pollination, predation, and competition) could be designed and applied, either in the field or the laboratory,

to provide information more suitable than that derived from "conventional" toxicity tests.

With the caveats described previously, the following general testing methods, with attendant advantages and disadvantages, might be appropriate for estimating ecological risk from contaminants, via atmospheric, water, or soil exposures, to certain terrestrial T&E species.

Plants

The major types of plant responses usually encountered in efficacy and phytotoxicity tests are described in ASTM's *Standard Guide for Evaluation of Nematode Control Agents–Plant Responses* (ASTM 1991). This guide is conceptually advantageous over pure phytotoxicity test standard operating procedures (SOPs) in that it emphasizes chemical influences on the vulnerability of plants to pathogens. Thus, the ASTM (1991) framework for assessing responses of plants to hazardous materials is broader than that in EPA's "terrestrial indicators" manual (EPA 1992b). Additional information may be found in PHYTOTOX, a database for phytotoxicity literature compiled at the University of Oklahoma (Fletcher, Johnson, and McFarlane 1988).

Exposure regimes to smoke munitions vary greatly in relation to terrain and convective conditions (Policastro et al. 1990). Thus, for estimating ecological risk to T&E species, use of *in situ* testing should be emphasized. Field-enclosure tests can be used to assess the effects of gaseous pollutants on photosynthate transport in plants such as soybean (e.g., Madkour and Weinstein 1988). However, if data-logging instruments are available to monitor exposure regimes to an atmospheric pollutant, exposure chambers might not be needed. In such cases, an *in situ* test could involve the use of one or more surrogate species in a series of paired plots (reference sites vs sites exposed to the contaminant(s) in question). Because variation in environmental conditions other than pollutants will occur, the paired plots should be selected to deliberately encompass a range of conditions (e.g., soil moisture, organic content, and exposure to sunlight) significant to the T&E plant species. Response parameters should include seed survival (which includes changes in seed viability and losses to seed predators), germination success, seedling growth, root development, and seed production.

Before investing significant effort in a field-scale *in situ* test, a screening test based on pollen viability should be considered for cases in which the T&E species are angiosperms or gymnosperms. The processes of pollen transportation and germination are central to reproductive success for many plant species, and pollen viability

can be tested easily and inexpensively using standardized laboratory procedures (Brewbaker and Kwack 1963, Young and Stranton 1990). Pollen viability also may be used as a response parameter in the *in situ* tests, for reasons of ecological significance and because of their relatively low cost. If the species to be protected is a bryophyte, laboratory tests analogous to those used to assess viability of pollen might be used to assess the inhibitory effects of pollutants on the flagellated male reproductive cells (cf. Morgan, Wu, and Young 1990).

Selection of plant species that are appropriate for use as surrogates for the T&E species in question must be considered carefully, but logistical considerations such as availability, size, and a short life cycle may override questions of sensitivity to pollutants, particularly if reproductive endpoints are used. Primarily for logistical reasons, but also because of its simple genomic structure, the small flowering plant *Arabidopsis thaliana* is gaining acceptance for use in toxicity tests of soils. A draft SOP for use of this plant species in laboratory tests to assess soil toxicity, with seed germination and shoot biomass as response variables, is included as Appendix B in this report. Procedures such as those described for soil testing with *A. thaliana* could be modified easily to include pollen viability measurements or be used to assess effects of atmospheric or waterborne pollutants. The small size and rapid life cycle of plants such as *A. thaliana* and *Tradescantia* spp. (note Schaeffer et al. 1987) are also attributes that can be used to advantage in *in situ* tests.

Soil Invertebrates

Although many kinds of soil-dwelling invertebrates have been or could be used in toxicity tests to provide information about ecological risk to T&E species (cf. EPA 1992b), earthworms are among the best. Earthworms are abundant and ecologically significant because they modify soil quality (by their foraging and tunneling) and are important components of the diets of many birds and small mammals. Earthworms also are readily exposed to contaminants in soil, both by ingestion and dermal contact; thus, contaminants that accumulate in earthworms can be readily transferred to their predators. Most earthworm species are large enough to assess individually but small enough for convenient testing. Various species, notably *Eisenia foetida*, have moderately short life cycles and can be reared easily in the laboratory. Finally, both laboratory test methods (e.g., Callahan, Russell, and Peterson 1985) and field testing methods (e.g., Callahan et al. 1991) have been developed for assessing the effects of soil contaminants on earthworms. These considerations account for the continued use of earthworms for estimating ecological risk from contaminants in soils.

Most of the published methods for testing the toxicity of soils with earthworms have used lethality as the measured endpoint. A recent study, however, shows that measurements of earthworm growth and reproduction can be used to reveal differences among soils in cases where no differences in survival of the earthworms were noted (Gibbs, Wicker, and Stewart 1994). Thus, the improvements in methodology suggested by this study should increase the usefulness of earthworm tests for assessing the biological effects of contaminants in soils. A draft SOP for conducting *E. foetida* tests of soils based on procedures described by Gibbs, Wicker, and Stewart (1994) is included as Appendix C in this report.

Although single-species assays (e.g., *E. foetida* test) are widely used to assess ecological impacts of contaminants in soils, they may not provide accurate estimates of pollution-induced changes in interspecies interactions. The disadvantages of single-species tests can be overcome by use of multispecies mesocosm tests. Soil mesocosm tests can be used to assess responses of various soil organisms such as microarthropods or soil nematodes, or soil-microbe processes (e.g., denitrification or decomposition) to contaminants (e.g., Parmalee et al. 1993; EPA 1992b; Gunderson et al. 1994). Multispecies mesocosm tests are advantageous in that they can permit insight into food-web structure and microbe-plant interactions of ecological significance. However, they tend to be more costly than single-species tests and may not use endpoints that are easily incorporated into an ecological risk-assessment framework. Because soil microarthropod communities are diverse and include a functional trophic web made up of predator and prey species, it is likely that soil-testing procedures such as those used by Parmalee et al. (1993) or recommended by EPA (1992b) will be used more often for ERAs.

Birds

Various bird species have been used to assess potentially adverse effects of chemicals in the environment, including ducks (Brewer et al. 1988), starlings (Grue, Powell, and McChesney 1982), kestrels (Rattner and Franson 1983), laughing gulls (White, Mitchell, and Hill 1983), and bobwhite quail (Galindo et al. 1984). The economic importance of birds commonly consumed by humans (e.g., chickens, turkeys, and ducks) is considerable and suggests that standardized toxicity tests with at least some species of birds may be used to assess chemicals of agricultural interest. An ASTM standard (E 857-87; ASTM 1994) for conducting subacute dietary toxicity tests with avian species is also available, but this guide is designed primarily for application to northern bobwhite, Japanese quail, mallard, and ring-necked pheasant. The result of tests conducted according to the ASTM standard "provides one basis for deciding whether additional toxicity testing should be

conducted with birds." Thus, the procedure at best should be considered to be a screening test, perhaps with limited application to ERA needs.

Mammals

Toxicity tests for mammalian wildlife have not been widely used in ERAs (EPA 1992b). Available methods consist of laboratory tests focusing primarily on small mammals (essentially rodents) and mustelids (mink and ferrets). *In situ* methods are not currently available. Standard methods for mammalian laboratory toxicity tests are summarized in EPA (1992b) and in ASTM (1994).

Reptiles

Various studies have demonstrated that contaminants or contaminant residues can be elevated in reptiles such as snakes (Fleet, Clark, and Plapp 1972) and turtles (Meyers-Schone and Walton 1994). However, relatively few studies have focused on the effects of contaminants on reptiles (Hall 1980). Additionally, no standardized toxicity test procedures appear to be available for this group of animals. In some habitats, reptiles are significant ecologically; lizards, for example, are important as predators of insects in arid or semiarid regions (cf. Pianka 1974). Many lizards also are territorial, have small home ranges, and can be kept in captivity easily. Thus, field or laboratory tests to assess the effects of contaminants on lizards probably could be conducted. These procedures, though, would likely be appropriate only for selected geographical areas.

Amphibians

An ASTM standard guide for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians (ASTM 1994) states that, for amphibians, "young larvae should be used whenever possible," and provides good general guidance for toxicity testing with aquatic phases of amphibians. The standard does not explain how to test terrestrial phases of amphibians (e.g., adult toads such as *Bufo americanus*) and does not provide a detailed methodology for testing egg or larval stages of amphibians. However, it does provide a strong set of references to reputable scientific studies that do provide necessary details for conducting both flow-through and static tests with amphibians.

Recent evidence for global reductions in species diversity of amphibians (Barinaga 1990) coupled with speculation about the possibility that this reduction is due at least in part to pollutants and increases in ultraviolet-B (UV-B) radiation has kindled interest in factors that influence amphibian abundance and species

richness. These studies may generate additional interest in developing amphibian-based toxicity test systems.

A frog embryo teratogenesis assay using *Xenopus laevis* has been used to estimate mutagenicity of chemicals. This test is referred to as FETAX and is reported to be a rapid, inexpensive system for preliminary assessment of potential developmental hazards (Dawson et al. 1988; Dawson, Schultz, and Baker 1991; Dumont et al. 1983). No other standardized toxicity test procedures appear to be available for this group of animals. In most instances, it would be reasonable to suppose that the most vulnerable life phase of an amphibian would be that associated with reproduction or juvenile development. Because these phases are aquatic for amphibians, toxicity test procedures for aquatic organisms probably would be adequate for estimating ecological risks from contaminants to amphibians.

Devillers and Exbrayat (1992) provide an extensive summary of existing field and laboratory toxicological studies on amphibians. References discussed in this volume may be useful both for toxicity data and for amphibian toxicity testing methods.

Proposed Methods

Most terrestrial toxicity test methods use single-species designs, involve short-term exposure regimes, and monitor organism growth or lethality as an endpoint. Most of these tests do not include significant ecological processes and exclude important species interactions that influence ecosystem trophodynamic structure. They also usually ignore the potential for sublethal effects of contaminants on reproductive success. Simple laboratory test methods could be developed to include quantitative consideration of predation as a trophic process and predator growth and reproductive success. This is in response to contaminant exposures via direct and indirect pathways, by use of readily available terrestrial invertebrates. Figure 5 shows an example of this possibility.

A test with the three units in Figure 5 could be extremely flexible. Exposures could consist of application(s) of the pollutant(s) in question to the soil, plants, and detritus only, either by atmospheric injection or by water; to the soil-plant-detritus component, in the presence of either or both prey and predator; or to all components, as desired. Prey items could be added at particular times after the contaminant(s) have been added to provide estimates of temporal changes in bioavailability of the contaminant. The predator, too, could be inserted into the system at a desired time after the prey have been added to permit several levels of prey contamination. Responses of the prey to the contaminant(s) could be quantified in terms of avoidance behavior and/or feeding activities. Sublethal

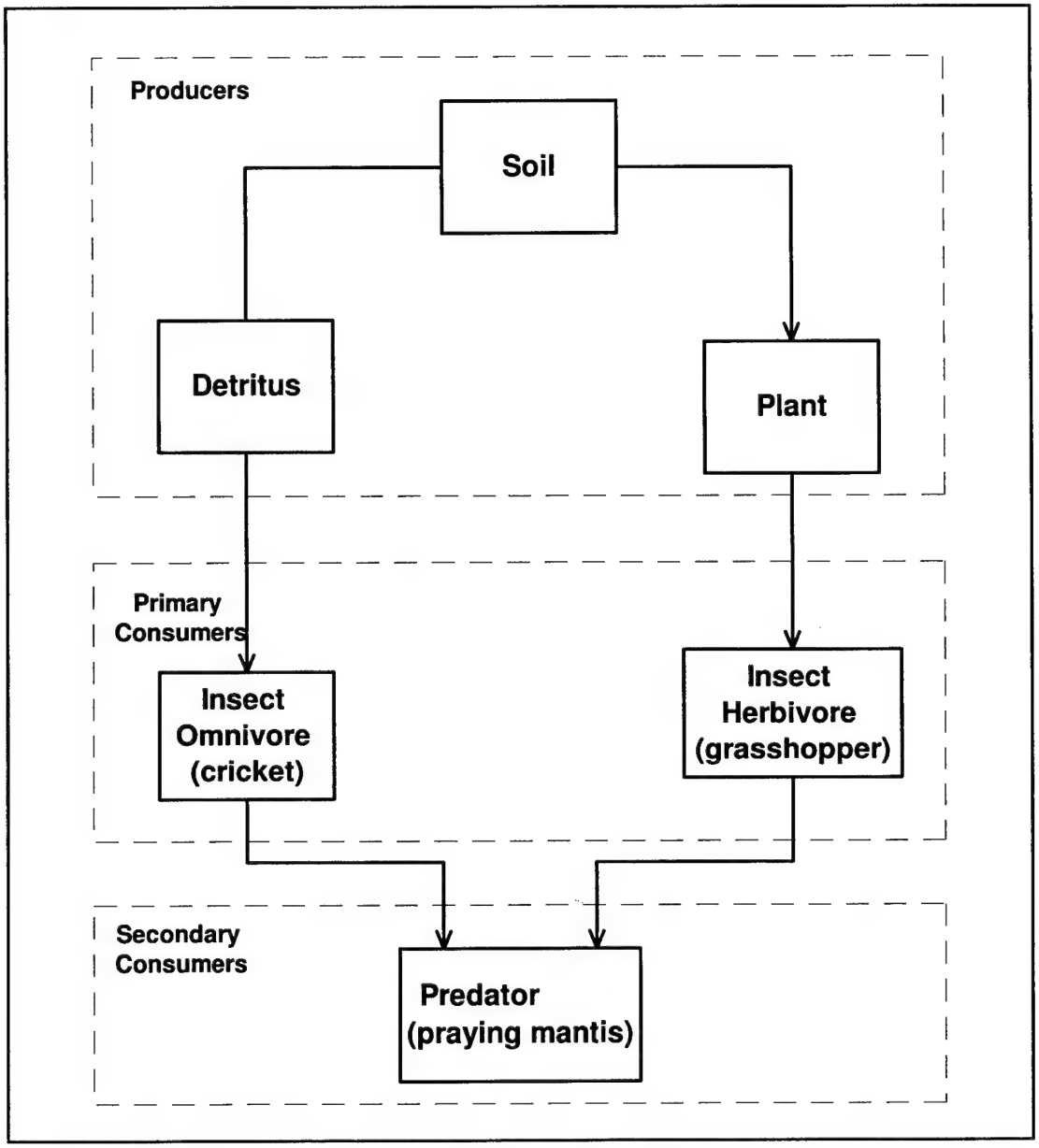

Figure 5. Trophic-level toxicity test for terrestrial invertebrates.

effects of the contaminants on the prey also could be inferred by assessing their ability to escape or delay predation. Sublethal effects of the contaminants on predator efficiency could be determined by strike/capture ratio, prey-handling time, or by direct measurements of predator growth. Because the life cycle of *Stagmomantis carolina* (the most common mantid in the southern United States) and other mantid species essentially terminates with the production of a conspicuous overwintering egg mass, second-generation effects of the exposure regimes could be followed (see Birchard 1991).

Various other arguments justify the consideration of insects in developing connective toxicity tests such as this. For praying mantis species, the size of the

predator and prey organisms is great enough so that the tests could be conducted easily in the field (see also Bartley 1982). The organisms also are small enough that the tests could be conducted easily in the laboratory. Praying mantis species and, presumably, other insects have strongly stereotypic movement behaviors that would permit easy analysis of behavioral abnormalities that might result from exposure to contaminants in air or their prey (Corrette 1990; Rossel 1986; Maldonado, Jafe, and Balderrama 1979). Additionally, within-brain physiological correlates to insect behaviors have been identified for the praying mantis, *S. biocellata*, suggesting that the physiological literature could support the development of clear linkages between exposures to contaminants and adverse effects. Insects are wonderfully diverse and enormously abundant; many species are critical components of the diets of T&E species of birds and small mammals or pollinators of T&E species of flowering plants. Finally, insect population responses to toxic chemicals have been well documented in the scientific literature because of the significance of insects to agriculture. Thus, many "field scale" studies of the responses of insect communities have already been conducted in support of the agricultural industry.

Toxicity Tests for Aquatic Biota

The application of toxicity tests for aquatic biota in risk assessment has developed from a total absence of this type of data (risk assessments were performed using only measured chemical concentrations to predict effects) to the development and use of standardized toxicity tests in ERAs. Standardized tests are favored because they are easy to conduct and the results can be replicated. Results from single-species tests are considered to be representative of impacts to broad classes of organisms (e.g., fish or invertebrates); the data provide information on the toxicity of specific chemicals to different types of organisms under given conditions (Rand and Petrocelli 1985).

In addition to single-species tests, tests may be performed using laboratory microcosms or model ecosystems. Laboratory microcosms are small-scale enclosures containing samples from the natural ecosystem (e.g., water, sediment, fish, invertebrates, and plants). One ASTM microcosm procedure (ASTM 1994), for example, is designed to obtain information concerning toxicity or other effects of a test material on the interactions among three trophic levels and the competitive interactions within each trophic level. The advantage of microcosms is that effects beyond the level of a single species can be identified. In principal, if conditions are uniform, these tests should be easy to replicate and standardize for different chemical substances; however, the literature indicates that this is not always the case (Rand and Petrocelli 1985).

Another type of test that may be used to evaluate effects to T&E species are *in situ* toxicity tests. *In situ* tests are performed by exposing test animals to conditions in a natural ecosystem. These tests may provide the most "real-world" assessment of toxicity. Use and interpretation of data from *in situ* tests, however, is limited because of a high degree of variation resulting from environmental conditions and the difficulty in establishing a cause-and-effect relationship. In addition, standard methods for *in situ* aquatic toxicity tests are not currently available. *In situ* tests could be developed on a site-specific basis, however.

The criteria for choosing an appropriate toxicity test for risk assessment may follow the recommendations of Rand and Petrocelli (1985):

1. The test should be widely accepted by the scientific community.
2. The test should be able to predict the effects of a wide range of chemicals on different organisms.
3. The test procedures should have a sound statistical basis and should be repeatable in different laboratories with similar results.
4. The data should include effects of a range of concentrations within realistic durations of exposure. They should also be quantifiable through graphical interpolation, which is an accepted method of quantitative evaluation.
5. The data should be useful for risk assessment.
6. The test should be economical and easy to conduct.
7. The test should be sensitive and as realistic as possible in design to detect and measure the effect.

Standard laboratory toxicity tests may measure acute or chronic effects to a variety of aquatic animals. Acute toxicity tests measure those effects that occur rapidly as a result of short-term exposure to a chemical(s). In fish and other aquatic organisms, effects that occur within a few hours, days, or weeks are considered acute (Rand and Petrocelli 1985). The most common acute effect measured is lethality. Lethal concentration 50 (LC_{50}) is the concentration that kills 50 percent or more of the exposed population of test organisms in a relatively short time, such as 96 hours to 14 days (Rand and Petrocelli 1985). Acute tests could be used to identify the concentration of a chemical, such as those found in smokes, which would cause a rapid effect on survival. If a reasonable aquatic community exists at the site in question, an acute test of ambient water impacted by smokes would provide little information. Acute tests described in the following may be conducted with aquatic invertebrates, fish, and aquatic insects.

Chronic or subchronic toxicity tests will provide the most information on specific chemicals and ambient water samples. The tests typically measure more sensitive

endpoints such as growth, reproduction, or emergence. Investigators at Oak Ridge National Laboratory have also been successful at applying standard toxicity tests to receiving waters (Kszos 1994, Stewart 1994). Chronic effects may occur when a chemical produces deleterious effects as a result of a single exposure, but more often they are a consequence of repeated or long-term exposures (Rand and Petrocelli 1985). For the same reasons stated in the discussion of acute tests, it is recommended that surrogate test species be used for testing rather than T&E species.

Appropriate duration of toxicity tests depends on (1) the species of interest, (2) the chemical (or receiving water) of interest, and (3) the criterion of concern. APHA (1989) divides laboratory toxicity tests into four categories: (1) short-term, acute toxicity tests, (2) intermediate toxicity tests, (3) long-term, partial- or complete-life-cycle toxicity tests, and (4) short-term tests for estimating chronic toxicity. The length of the organisms's life cycle helps determine what is short-term, intermediate, or long-term. In short-term definitive tests, lethality is the most common endpoint. Tests may be static, static-renewal, recirculation, or flow-through. Exposure periods for these tests usually are 48 or 96 hours. Intermediate tests generally last for 11 to 90 days and flow-through is recommended, although they may be static or renewal (APHA 1989). Long-term, partial- or complete-life cycle tests are nearly always flow through and extend over as much of the life cycle as possible. Tests are continued from egg to egg or up to several life cycles for smaller animals.

In risk assessment, standard single-species tests are emphasized because data are readily available for many chemical compounds, and they can be readily conducted by testing laboratories (Suter 1993). Representative standard test methods are listed in the following with a brief description of their applicability. The primary sources for standard methods are (1) EPA, (2) ASTM, and (3) the American Public Health Association (APHA). SOPs for some test methods are supplied in Appendix D and so indicated in the following sections.

Aquatic Invertebrates

Invertebrates are an important component of lake and stream ecosystems and are a major food item for many species of fish. Several standard tests are available for measuring the acute toxicity of a chemical or ambient water to invertebrates. The most commonly used are the 24- to 96-hour tests with microcrustaceans, *Ceriodaphnia* sp. or *Daphnia* sp. (Weber 1993; APHA 1989; ASTM 1994; Appendix D); EPA methods tend to be the most detailed. The disadvantage of these tests is that the test species typically live in lakes and ponds and may not be representative of stream or river invertebrates. However, the animals are easy to culture, the tests are well standardized, data are readily available on the acute toxicity of many

chemicals to both species, and both species are readily available throughout the year from commercial sources.

If the benthic community surveys identify an impacted invertebrate community, several additional tests using invertebrates or aquatic insects should be considered. APHA (1989) provides a description of test methods for amphipods, isopods, and crayfish that measure acute toxicity. Because these animals have more direct contact with the substrate of the stream or pond, they are better surrogates for aquatic insects than *Ceriodaphnia* or *Daphnia*. APHA (1989) also has a standard method for testing with stoneflies, mayflies, caddisflies, and diptera. Toxicants may interfere with survival, growth, reproduction, emergence, and metabolism of aquatic insects. Because effects of long-term exposure to sublethal concentrations of toxicants may be more important than effects of infrequent short-term exposure to higher concentrations, flow-through, long-term tests are recommended (APHA 1989).

Methods to measure chronic toxicity to invertebrates have been standardized and, again, the most commonly used test species are the microcrustaceans *Ceriodaphnia dubia* (Weber et al. 1989; APHA 1989; ASTM 1994; Appendix D) and *Daphnia* sp. (Weber et al. 1989, APHA 1989). These tests are easily adapted to assess ambient toxicity or chemical-specific toxicity. It is advisable to use these tests, rather than the acute tests, because they measure effects on reproduction, which is usually more sensitive than lethality. The test methods identified above for aquatic insects and amphipods may also be used for life-cycle studies. Such tests would be advisable if the benthic community is impacted, but no effects are observed using the acute or short-term chronic tests with microcrustaceans.

Fish

Standardized toxicity tests with fish are described in ASTM (1994), APHA (1989), and Weber (1993). Fathead minnows are the most commonly used test species, although methods may be adapted to many other species. Acute test methods measure the effect of a toxicant or ambient water on survival. As with the invertebrate tests, a survey of the community present at the site of interest would indicate whether an acute test would provide additional information. Acute tests are best used to determine the toxicity of a specific chemical.

Chronic toxicity tests with fish measure effects such as growth, egg viability and hatchability, and development. The advantage of using these tests for ambient samples is that they measure a more sensitive endpoint than mortality. A commonly used, short-term chronic test is the EPA fathead minnow survival and

growth test (Weber et al. 1989). These minnows are typically less sensitive than *Ceriodaphnia* to contaminants in ambient waters, although this is not always the case (Kszos 1994). ASTM (1994) and APHA (1989) provide suitable guidance for conducting tests with freshwater fish such as sunfish, minnows other than fathead minnows, salmon, and perch. If the T&E species of concern is not a minnow, a test with a surrogate species that is taxonomically more related should be considered.

Multiple-Species Tests

Aquatic microcosms containing multiple species can be used to estimate the effects of a chemical on the dynamics and metabolism of an ecosystem. ASTM (1994) provides a standard practice (E 1366) for conducting tests with aquatic microcosms. The practice covers procedures for obtaining data concerning toxicity of a test material to a multitrophic-level freshwater community consisting of substrate, algae, and *Daphnia.* Limitations include: (1) no fish or other vertebrate is included, (2) the ecosystem becomes nutrient limited, and (3) predation on *Daphnia* is absent. ASTM (1993) also published a standard guide (E 1197-87) for conducting a terrestrial soil-core microcosm test. Using a special extraction tube, the microcosm is collected from an appropriate natural source as an intact core and contains a natural assemblage of soil organisms and plants. However, the only terrestrial animals included are small invertebrates. Additional microcosm studies can be found in the literature and could be designed for T&E species.

Sediment Biota

Many of the same criteria used for selecting a toxicity test method for water can be used for selecting a sediment test method. Sediment testing, however, is only just beginning to become standardized, with the first EPA draft methods published in 1994. The EPA has standardized three whole-sediment tests. Two of these tests are short-term (they last for 10 days); one is long-term and measures bioaccumulation. Current research needs for sediment testing include: (1) chronic sediment toxicity tests, (2) selection of additional test organisms, (3) development of formulated sediments, (4) sediment spiking, and (4) field validation of laboratory tests (EPA 1994b).

The 10-day standard sediment test uses an amphipod, *Hyalella azteca* (EPA 1994b; ASTM 1994; Appendix D) and a midge, *Chironomus tentans* (EPA 1994b; ASTM 1994). According to the EPA (1994b), advantages of these test species include: (1) relative sensitivity to contaminants associated with sediment, (2) short generation time, (3) contact with sediment, (4) ease of culture in the laboratory, and (5) tolerance to varying physicochemical characteristics of sediments. The test with an

aquatic earthworm (*Lumbriculus variegatus*) can be used to measure toxicity and bioaccumulation of sediment-associated contaminants. These tests might be appropriate if it were suspected that chemicals from the smokes had accumulated in the sediment.

In Situ and Nonstandard Test Methods

Another type of test that may be used to evaluate effects to T&E species are *in situ* toxicity tests. *In situ* tests are performed by exposing test animals to conditions in a natural ecosystem. These tests may provide the most "real-world" assessment of toxicity. Use and interpretation of data from *in situ* tests, however, is limited due to a high degree of variation resulting from environmental conditions and the difficulty in establishing cause-and-effect relationships. In addition, standard methods for *in situ* aquatic toxicity tests are not currently available. *In situ* tests could be developed on a site-specific basis, however. *In situ* tests that may be adapted to evaluate risks to T&E species include methods for sediment (Krantzberg 1992; Burton 1991), fish (Ziegenfuss et al. 1990; Hall et al. 1988), clams (Belanger 1991), benthic invertebrates (Whaley, Garcia, and Sy 1989), snails (Burris, Bamford, and Stewart 1990; Hinzman 1994 [see Appendix D]), amphipods (Crane and Maltby 1991), and *Ceriodaphnia* (Sasson-Brickson and Burton 1991).

9 Characterization of Risk to Threatened and Endangered Species

Risk characterization estimates risks by combining information concerning exposure to contaminants with information concerning effects of contaminants. Risk characterization for ERAs is performed by weight of evidence (EPA 1992a). That is, rather than simply modeling risks, ecological risk assessors examine all available data from chemical analyses, toxicity tests, biological surveys, and bioindicators (if available) to estimate the likelihood that significant effects are occurring or will occur and to describe the nature, magnitude, and extent of effects on the designated assessment endpoints. This chapter describes an approach for estimating risks that smokes and obscurants present to T&E species. The approach is based on individual lines of evidence that are combined through a weight-of-evidence process. As stated in Chapter 2, because of the limited populations and endangered status of T&E species, preventing adverse impacts on the *individual* is the focus of ERA for T&E species.

Single Chemical Toxicity

This line of evidence uses analyses of smoke residues in biotic or abiotic media to estimate exposure. It also uses literature values for effects of these chemicals to estimate effects to an individual of a T&E species (Figure 6). These uses are combined in two steps. First, the contaminants are screened against ecotoxicological benchmarks and background exposure. Contaminants for which exposure exceeds benchmarks and background exposure (if applicable) are identified as contaminants of potential concern (COPCs).

For contaminants identified as COPCs, the second step is to compare exposures with the full toxicity profile of the contaminant to characterize risk. For example, the distribution of concentrations in water would be compared with the distribution of concentrations of thresholds for chronic toxicity across fish species and across prey species. The nature of the chronic effects would be described, and the exposure durations needed to achieve effects in the laboratory would be compared with temporal dynamics of concentrations in the field. Characteristics of the contaminants

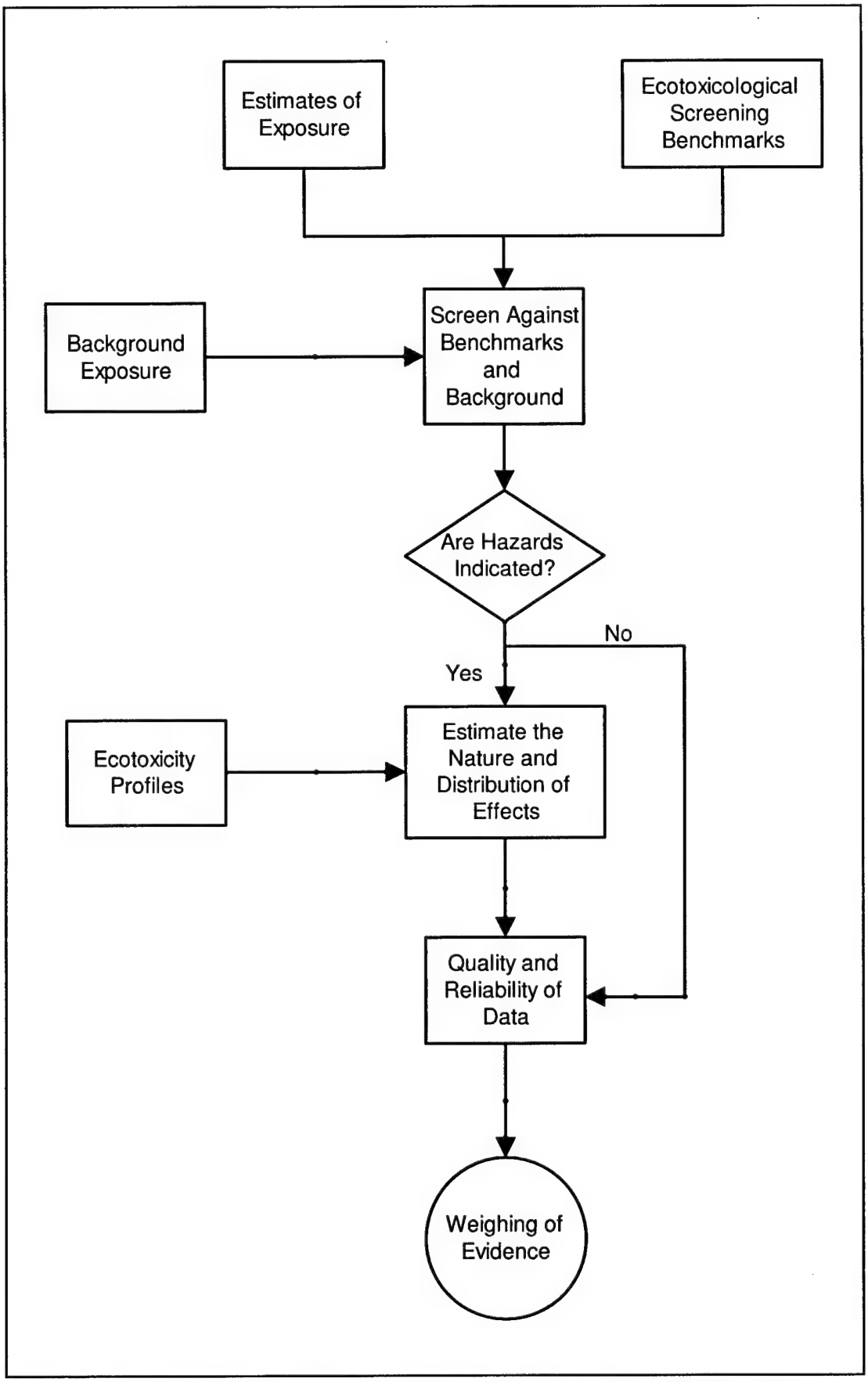

Figure 6. Risk characterization based on chemical analyses and single chemical toxicity.

that are relevant to risks are also examined, such as the influence of metal speciation on toxicity, tendency of the contaminant to accumulate in prey species, etc.

The result of risk characterization for this line of evidence should be statements about the following questions:

- Are toxic concentrations of contaminants present?
- What effects do these concentrations cause in the laboratory or at well-studied sites?
- How extensive are toxic concentrations relative to the range of the receptors?
- How long do toxic concentrations persist relative to the time required for effects to occur?
- How frequently do toxic concentrations occur relative to the recovery time of the receptors?
- Are they associated with identifiable sources?
- How much must the source be diminished to eliminate toxicity?
- How much confidence is there in the answers to these questions?

Ambient Media Toxicity Tests

Risk characterization for this line of evidence begins by determining whether the tests show significant toxicity (Figure 7).

- If no significant toxicity was found, the risk characterization consists of determining the likelihood that the result constitutes a false negative. False negatives could result from (1) not collecting samples from the most contaminated sites or at the times with the highest contaminant levels, (2) handling the samples in a way that reduced toxicity, or (3) using tests that are not sufficiently sensitive to detect effects that would cause significant injuries to populations or communities in the field.
- If significant toxicity occurs in the tests, the risk characterization should describe the nature and magnitude of the effects and the consistency of effects among tests conducted with various species in the same medium.
- Toxicity tests may yield ambiguous results in some cases because of poor performance of organisms in control media (e.g., resulting from diseases, background contamination, inappropriate reference or control media, or poor performance of the test protocol). In such cases, expert judgement by the assessor in consultation with the individuals who performed the test should be used to arrive at an interpretation of the test results.

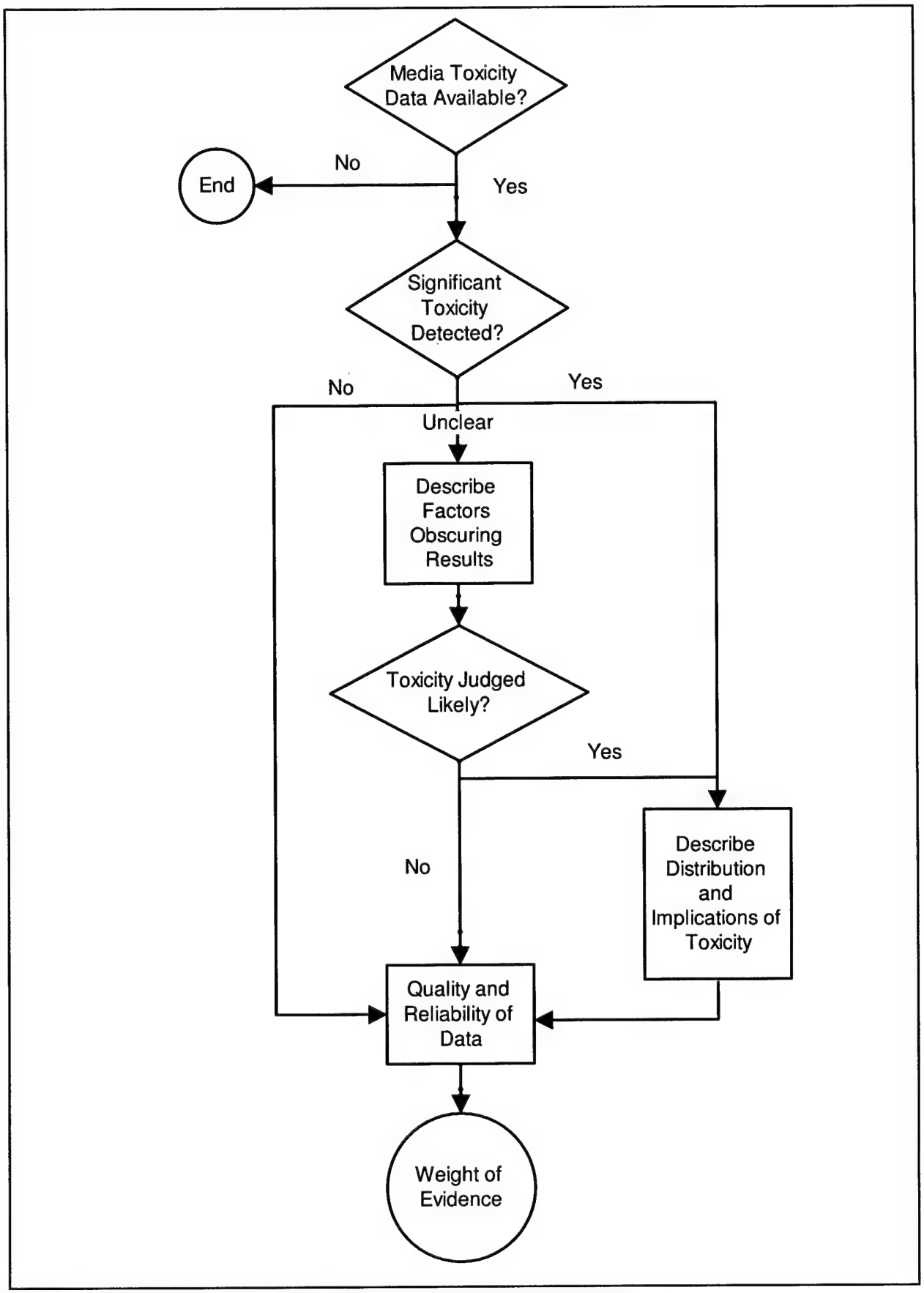

Figure 7. Risk characterization based on toxicity testing of ambient media.

If significant toxicity is found at any site, the relationship of toxicity to exposure must be characterized. The first way to do this is to examine the relationship of toxicity to concentrations of contaminants in the media. The manner in which this is done will depend on the amount of data available. If numerous toxicity tests are available, the frequency of tests showing toxic effects could be defined as a function of concentrations of one or more COPCs (Stewart et al. 1994). An alternative and potentially complementary approach is to determine the relationship between the occurrence of toxicity and the spatial distribution of smoke residues.

The result of risk characterization for this line of evidence should be statements about the following questions:

- Is toxicity occurring?
- How severe is it?
- How extensive is toxicity relative to the range of the receptors?
- How frequent is toxicity relative to the recovery time of the receptors?
- Is it associated with identifiable sources or contaminants?
- How much must the source be diminished to eliminate toxicity?
- How much confidence is there in the answers to these questions?

Biological Surveys

If biological survey data are available, the first question to be answered is whether the data suggest the occurrence of significant effects (Figure 8). This is determined through statistical comparisons of data from the smoke-exposed site(s) to those from the reference sites.

If biological survey data are consistent with significant reductions in abundance, production, or diversity, associations of apparent effects with causal factors must be examined. First, the distribution of apparent effects in space and time must be compared with the distribution of sources or of contaminants. Second, the distribution of apparent effects must be compared with the distribution of habitat factors that are likely to affect the organisms in question such as stream structure and flow. Finally, the natural variability of the endpoint populations and communities and the accuracy of the survey methods must be examined to estimate the likelihood that the apparent effects are due to chance.

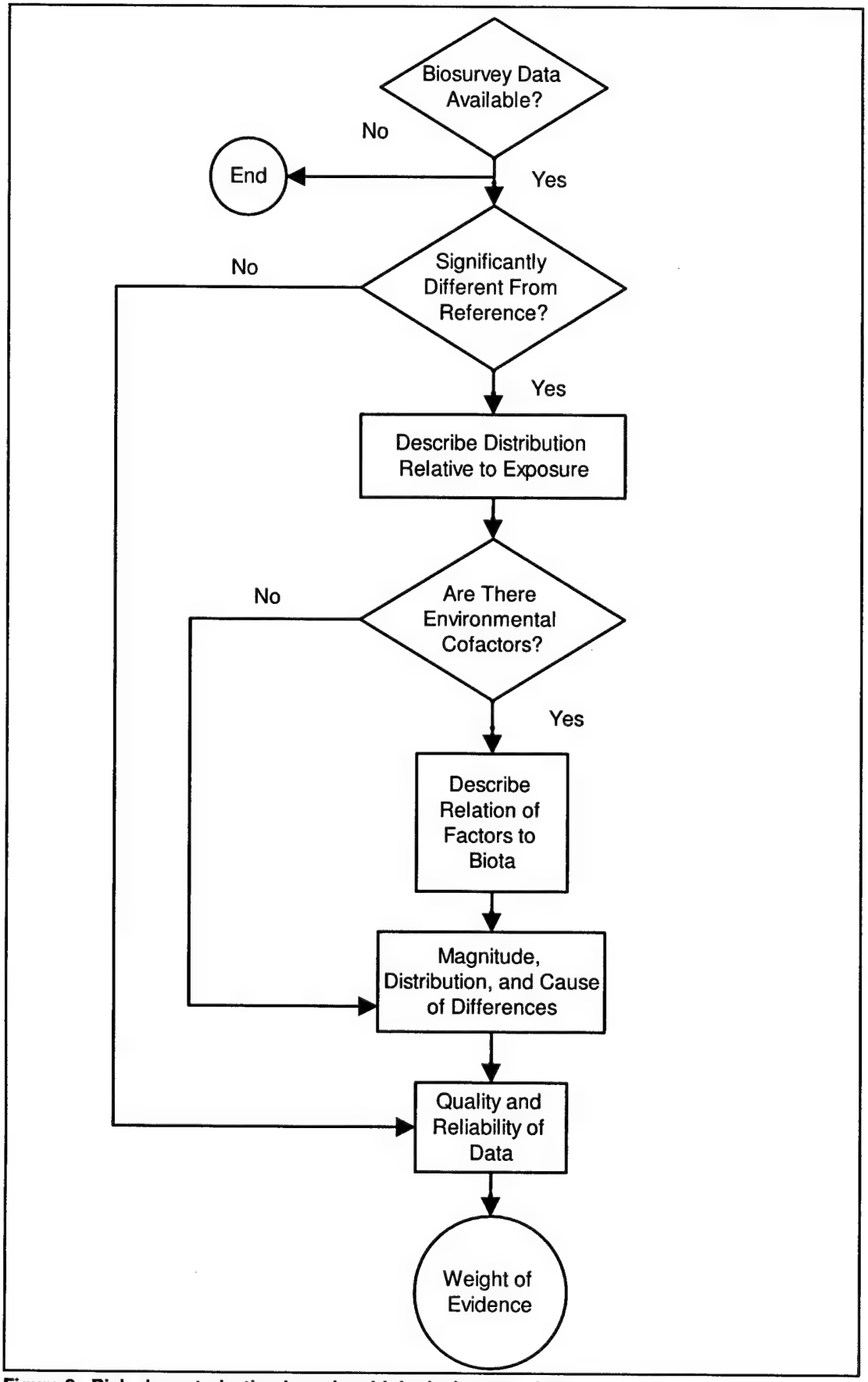

Figure 8. Risk characterization based on biological survey data.

The result of risk characterization for this line of evidence should be statements about the following questions:

- Are the endpoint ecological properties significantly reduced?
- How much are they reduced?
- How extensively are they reduced?
- Is the reduction associated with identifiable sources of contaminants?
- Is the reduction associated with identifiable habitat variables?
- What is the most likely cause of the apparent reduction?
- How much confidence is there in the answers to these questions?

Bioindicators and Biomarkers

Bioindicators and biomarkers are biochemical or physiological changes that indicate that an organism has received an internal dose of a chemical. Examples include contaminant body burdens, histopathological observations, and measures of detoxification or stress enzyme levels. Bioindicators have not been discussed previously in this document because of the uncertainty concerning their availability for smokes and obscurants. If appropriate bioindicators are identified, methodologies for their use are described below.

Biological indicators are seldom useful for estimating risks by themselves, but they can be used to support other lines of inference. The inference begins by asking if the levels of the bioindicators differ significantly from those at reference sites (Figure 9). If they do, then it is necessary to determine whether they are diagnostic or at least characteristic of any of the COPCs or of any of the habitat factors that are thought to affect the biota in question. If the bioindicators are characteristic of contaminant exposures, the distribution and frequency of elevated levels must be compared with the distributions and concentrations of contaminants. Finally, to the extent that the bioindicators are known to be related to overt effects such as reductions in growth, fecundity, or mortality, the implications of the observed bioindicator levels for individuals or populations of the T&E species should be estimated.

The result of risk characterization for this line of evidence should be statements about the following questions:

- Are bioindicator levels significantly elevated?
- What are the implications for individuals?
- How extensive are the effects?

- Are they spatially or temporally associated with identifiable sources of contaminants?
- Are they spatially or temporally associated with identifiable habitat variables?
- Are they diagnostic or characteristic of a contaminant or a habitat variable?
- What is the most likely cause of the observed levels?
- How much confidence is there in the answers to these questions?

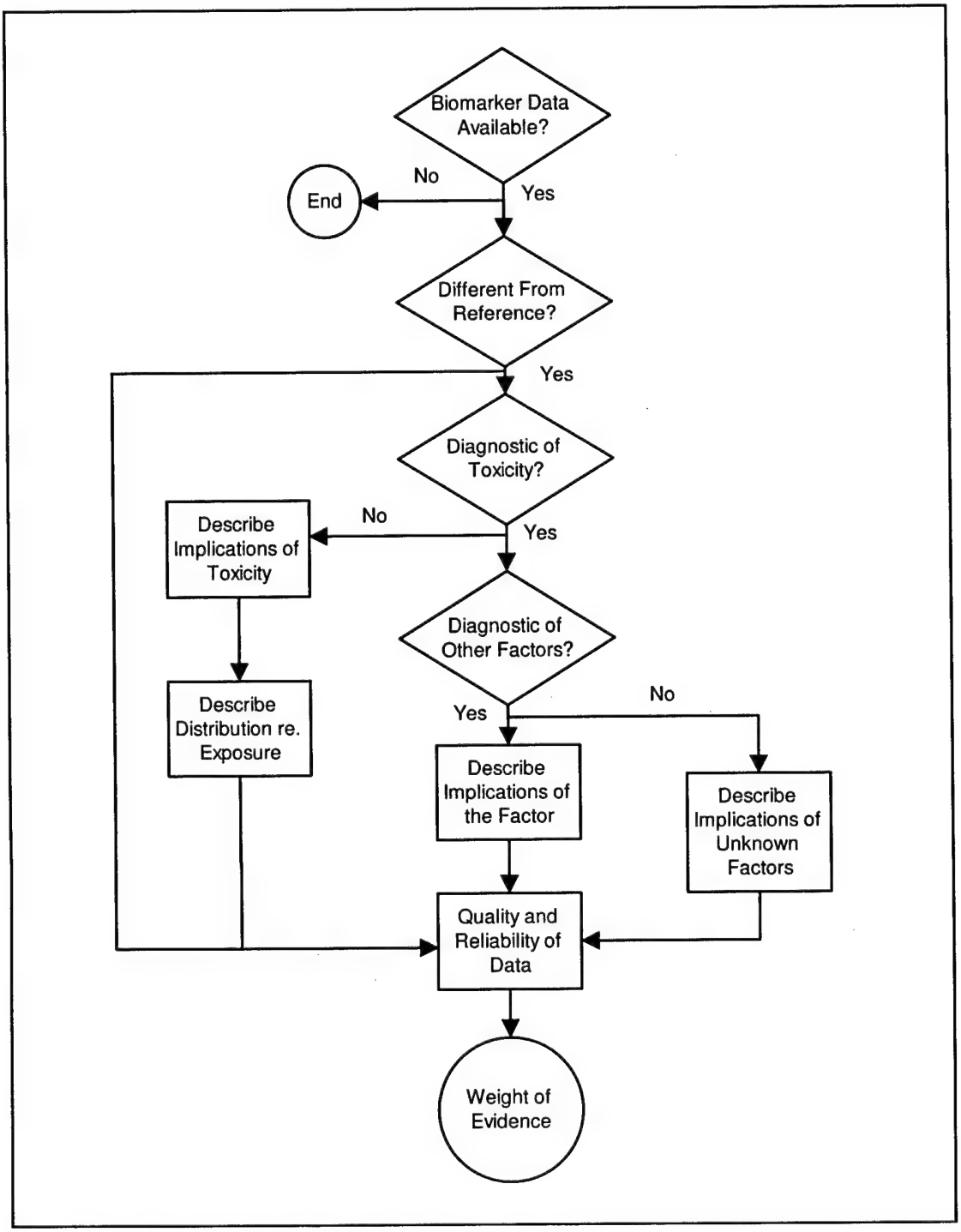

Figure 9. Risk characterization based on biomarker data.

Weight of Evidence

The weighing of evidence begins by summarizing the available lines of evidence for each endpoint (Figure 10). The tabular format presented in Table 5 is recommended. The lines of evidence are listed and a symbol assigned for each: + if the evidence is consistent with significant effects on the endpoint, - if it is inconsistent with significant effects, ± if it is too ambiguous to assign to either category, and NA if data for that line of evidence are unavailable. The last column presents a short summary of the results of the risk characterization for that line of evidence. If indirect effects are part of the conceptual model, they should be summarized in their own line of the table. For example, effects on the fish community could result entirely or in part from toxicity to invertebrate prey species. The last line of the table presents the weight-of-evidence-based conclusion concerning whether

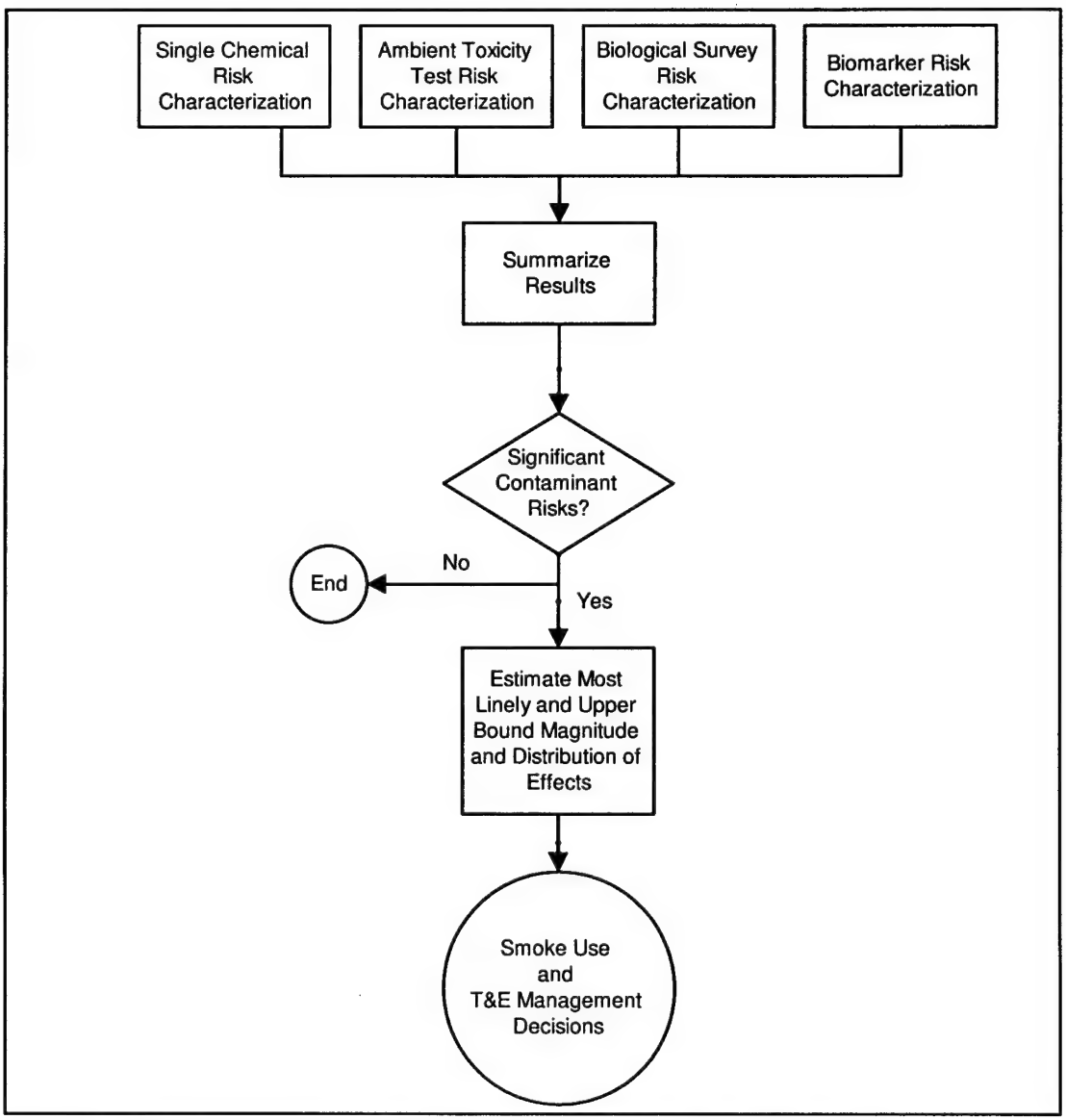

Figure 10. Risk characterization based on weighing of multiple lines of evidence.

Table 5. Example of a table summarizing the risk characterization for the fish community in a stream at a smoke-impacted site.

Evidence	Result*	Explanation
Biological Surveys	-	Fish community productivity and species richness are both high in reaches 2 and 3. Smoke residues apparently improve community quality.
Toxicity Tests	±	High lethality to fathead minnow larvae in a test in reach 3.3, but variability is too high for standard statistical significance.
Media Analyses	+	Only zinc is believed to be potentially toxic in water and only to highly sensitive species.
Weight-of-Evidence	-	Reaches 2 and 3 support a clearly high quality fish community. Other evidence that suggests toxic risks is much weaker.

* + indicates that the evidence is consistent with the occurrence of the endpoint effect.
- indicates that the evidence is inconsistent with the occurrence of the endpoint effect.
± indicates that the evidence is too ambiguous to interpret.

significant effects are occurring and a brief statement concerning the basis for the conclusion. This conclusion is not based simply on the relative number of + or - signs. The "weight" component of weight of evidence is the relative credibility and reliability of the conclusions of the various lines of evidence.

In general, the weighing of evidence is best accomplished by beginning with the line of evidence that most directly bears on the actual risks. That is, begin with the risk characterization based on biological survey data, if available. If, for example, the fish community is depauperate downstream of a source, check the risk characterization based on toxicity data to see if it indicates that aqueous toxicity is responsible. Check the bioindicators to see if the fish populations that are still present bear signs of suborganismal effects. Finally, look to the risk characterization based on analysis of media to determine what contaminants are likely to be responsible for any observed effects or toxicity. This process clearly relies on expert judgement, but that judgement should be presented as clearly as possible to the stakeholders.

If no significant effects are believed to be occurring, the assessment of that particular endpoint is complete. However, if significant effects are occurring, they must be characterized. That is, the nature, magnitude, and extent of the effects must be estimated. This estimation may also be based on multiple lines of evidence. Various lines of evidence may indicate that a significant effect is occurring but may disagree about its magnitude or extent. In general, the estimates will be based on the best evidence; that is, the evidence that provides the clearest and most accurate estimate of effects.

Uncertainties

Uncertainties should have been identified in the risk characterizations for each line of evidence, but the risk characterization should also include a summary of uncertainties and their implications. The Risk Assessment Forum (EPA 1992a) indicates that this discussion should include uncertainties resulting from the conceptual model formulation, incompleteness of information, stochasticity (natural variability), and error. Results of quantitative uncertainty analyses should be presented here, but it is important to remember that such analyses do not include all uncertainties. In particular, although it is possible to quantitatively estimate the uncertainty associated with a single line of evidence, it is not possible to quantify the total uncertainty associated with a conclusion reached by weighing multiple lines of evidence.

It is important to summarize the implications of the listed uncertainties. This summary should include:

- the credible maximum and minimum levels of effects
- endpoints that were not addressed
- routes of exposure or indirect modes of action that were not addressed
- conditions that were not addressed (e.g., storm events).

Example: Risk Characterization for Ospreys of the Clinch River/Poplar Creek System, Tennessee

To illustrate the application of risk characterization, an example was extracted from the Remedial Investigation/Feasibility Study for the Clinch River/Poplar Creek Operable Unit (DOE 1995) adjacent to the Oak Ridge Reservation (ORR). This example shows how conflicting lines of evidence are weighed, uncertainties are considered, and the likelihood of adverse impacts is estimated. Because this example is intended to show how to weigh evidence and perform a risk characterization, the results of the data analysis are only briefly summarized. The complete ecological risk assessment is presented in DOE (1995).

Background

ORR is on the Clinch River in East Tennessee, approximately 30 miles west of Knoxville. Through over 50 years of operations, activities at the three plants on the reservation (Oak Ridge National Laboratory, the Y-12 Plant , and the K-25 Plant) have released contaminants (primarily mercury, PCBs, and radionuclides) into the

Clinch River/Poplar Creek system. A risk assessment was performed to determine if these contaminants present a hazard to piscivorous wildlife. A weight-of-evidence approach was used to evaluate effects on ospreys (*Pandion haliaetus*). Data consisted of the contaminant concentrations in fish and water, and observations of reproductive success at osprey nests adjacent to the ORR. Consequently, available lines of evidence were limited to a comparison of contaminant exposure estimates to single chemical toxicity data and field surveys. Ambient media toxicity tests and bioindicators were not available.

Single Chemical Toxicity Data

Contaminant exposure experienced by ospreys was estimated using the fish and water contamination data. Both point estimates of exposure (derived using the upper 95 percent confidence interval on the mean contaminant concentration in fish and in water) and distributions of exposure (derived using a Monte Carlo simulation*) were generated. The exposure estimates using point estimates of parameter values at each individual sampling point were used to identify contaminants of potential ecological concern (COPECs) and locations that contributed significantly to risk. In contrast, the exposure distributions generated by Monte Carlo simulation represent the likelihood that an individual within the area for which exposure is modeled will experience a particular exposure.

Two types of single chemical toxicity data are available with which to evaluate exposure of ospreys to contaminants: no observed adverse effects levels (NOAELs) and lowest observed adverse effects levels (LOAELs). These values were obtained from Opresko et al. (1994). NOAELs are used to screen exposure estimates generated from point estimates of exposure parameters; if the estimate is greater than the NOAEL, adverse effects are possible and additional evaluation is necessary (i.e., exposure modeling using Monte Carlo simulation). LOAELs are compared to the exposure distribution generated by the Monte Carlo simulation. If the LOAEL is lower than the 80th percentile of the exposure distribution, there is a >20 percent likelihood that individuals within the modeled location are experiencing contaminant exposures that are likely to produce adverse effects. By combining literature-derived population density data with the likelihood or probability of exceeding the LOAEL, population-level impacts may be estimated.

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* Monte Carlo simulation is a resampling technique frequently used in uncertainty analysis in risk assessment. In practice, distributions are assigned to input parameters in a model and the model is recalculated many times to produce a distribution of output parameters (e.g., estimates of contaminant exposure). Each time the model is recalculated, a value is selected from within the distribution assigned for each input parameter. As a result, a distribution of exposure estimates is produced that reflects the variability of the input parameters.

Screening point estimates of exposure. To determine if the contaminant exposures experienced by ospreys along the Clinch River/Poplar Creek are potentially hazardous, which contaminants represent the hazard, and where this hazard is present, total contaminant exposure estimates were compared with estimated NOAELs. To quantify the magnitude of hazard, a hazard quotient (HQ) was calculated where: HQ = exposure/NOAEL. HQs greater than 1 indicate that individuals may be experiencing exposures that are in excess of NOAELs and suggest that adverse effects may be occurring.

The spatial distribution of contamination and potential risks to ospreys in the Clinch River/Poplar Creek system is illustrated in Figure 11. These distribution figures display the sum of the NOAEL-based HQs for the six most important contaminants: arsenic, copper, DDT, mercury, selenium, and total PCBs. (The quotient of an exposure divided by a toxicological benchmark may be thought of as an expression of the toxicological hazard or as toxicity normalized concentration or toxic unit [TU]. The TUs may be summed as in Figure 11 as an indication of relative potential toxicity.) Importance of contaminants was determined based on the magnitude of the HQ. River subreaches were arranged from the northernmost to the southernmost. The maximum ΣTU was observed at the Poplar Creek subreaches (13, 3.01, 3.02, 3.03, and 3.04). The contaminants contributing the most to total risk are mercury followed by total PCBs (Figure 11).

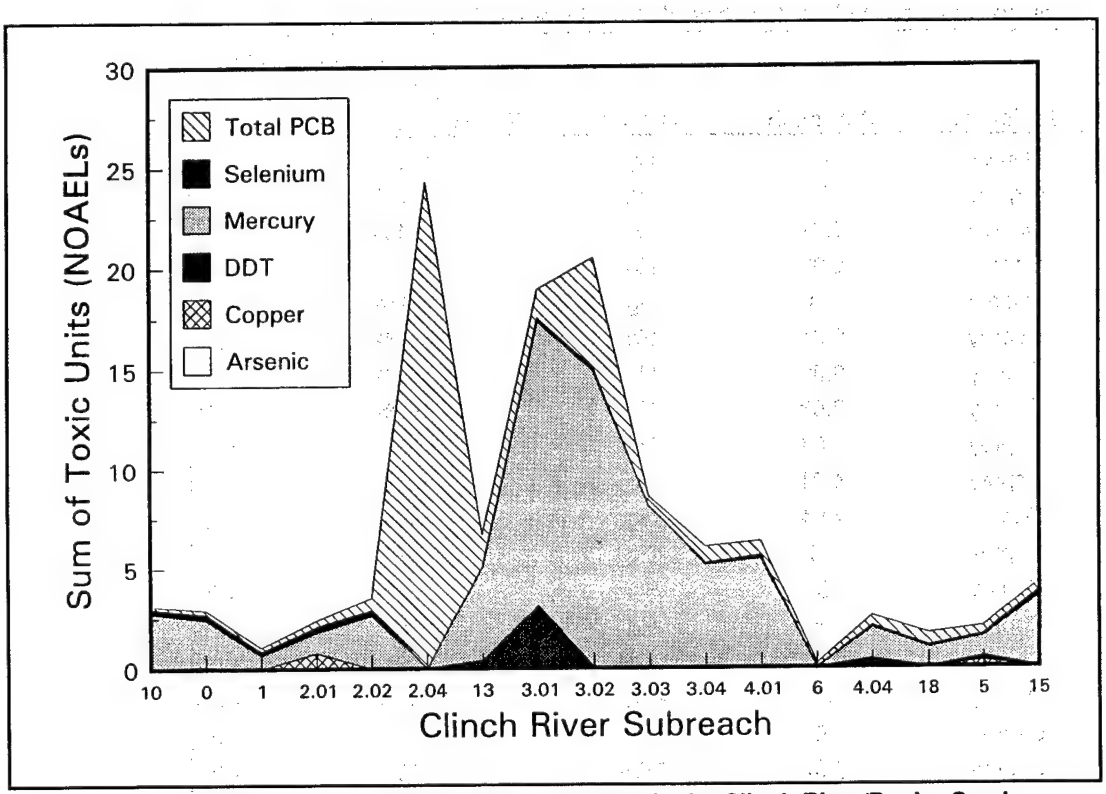

Figure 11. Contamination and potential risks to ospreys in the Clinch River/Poplar Creek system.

Screening Monte Carlo simulation estimates of exposure. To incorporate the variation present in the parameters used in the exposure model, Monte Carlo simulations were performed for exposure to contaminants where NOAEL-based HQs>1 were observed. By superimposing NOAEL and LOAEL values on these distributions, the likelihood of an individual experiencing potentially hazardous exposures can be estimated and the magnitude of risk to individuals may be determined. These comparisons are presented in Table 6. Table 7 is an interpretation of the comparison of exposure distributions to NOAELs and LOAELs.

Ospreys at subreaches 3.01 and 3.02 are estimated to receive exposures to mercury in excess of the LOAEL >99 percent and 70 percent of the time (Table 6). No other contaminants in any other subreach are estimated to present a risk to ospreys.

To accurately evaluate the significance of mercury exposure among ospreys within subreaches 3.01 and 3.02, the foraging range of ospreys must be considered. Ospreys are a wide-ranging species, with individuals ranging as far as 10 to 15 km from their nest sites in search of food (Van Daele and Van Daele 1982). EPA (1993a) reports the mean foraging radius for ospreys to be 1.7 km with a range of 0.7 km to 2.7 km. Of the three active osprey nests in the vicinity of the ORR (B. Anderson, personal communication), two are located along Melton Hill Reservoir

Table 6. Comparison of Monte Carlo simulation of piscivore contaminant exposure estimates to literature-based NOAELs and LOAELs.

			Within Subreach Exposure Estimate	
Endpoint	Subreach	Analyte	% > NOAEL	% > LOAEL
Osprey	10	Hg	>99%	<1%
Osprey	0	Hg	>99%	<1%
Osprey	2.01	Hg	<1%	<1%
Osprey	2.02	Hg	>99%	<1%
Osprey	13	Hg	>99%	<1%
Osprey	3.01	Hg	>99%	>99%
Osprey	3.02	Hg	>99%	70%
Osprey	3.03	Hg	>99%	1%
Osprey	3.04	Hg	>99%	<1%
Osprey	4.01	Hg	>99%	<1%
Osprey	4.04	Hg	>99%	<1%
Osprey	18	Hg	>99%	<1%
Osprey	5	Hg	>99%	<1%
Osprey	15	Hg	>99%	<1%
Osprey	2.04	PCB	>99%	<1%
Osprey	13	PCB	<1%	<1%
Osprey	3.01	PCB	<1%	<1%
Osprey	3.02	PCB	90%	<1%
Osprey	3.01	DDE	>99%	<1%

Table 7. Interpretation of the exposure distribution comparison to NOAELs and LOAELs.

Comparison	Meaning	Risk-based Interpretation
NOAEL>80th percentile of exposure distribution	Less than 20% of exposures> NOAEL	Individual- and population-level adverse effects are highly unlikely
NOAEL<80th percentile<LOAEL	More than 20% of exposures > NOAEL, but less than 20% of exposures> LOAEL	Individuals experiencing exposures at the high end of the distribution may experience adverse effects, but those effects are unlikely to significantly contribute to effects on the ORR population.
LOAEL<80th percentile of exposure distribution	More than 20% of exposures> LOAEL	Effects on some individuals are likely, and they may contribute significantly to effects on the ORR population.

(subreach 1) and one near K-25 on Poplar Creek approximately at the border between subreaches 3.03 and 3.04. While the Melton Hill Reservoir (subreach 1) nest sites are within 15 km of subreaches 3.01 and 3.02, due to the availability of suitable habitat nearer to their nests and that the mean foraging radius is 1.7 km, birds from these nest sites are unlikely to forage within subreaches 3.01 and 3.02. Therefore, potentially deleterious mercury exposure to these birds is unlikely. In contrast, the 3.03/3.04 nest site is within 3 km of subreaches 3.01 and 3.02. Birds from this nest may therefore forage and be exposed to elevated mercury in fish from subreaches 3.01 and 3.02.

To estimate and model the potential mercury exposure for ospreys at the 3.03/3.04 nest location, it was assumed that the birds would travel up to 5 km from the nest to forage. Most foraging was assumed to occur near to the nest, with approximately 50 percent of their diet obtained from within 1 km of the nest, 25 percent obtained from 1 to 2 km, 15 percent from 2 to 3 km, and 5 percent each from 3 to 4 km and 4 to 5 km from the nest, respectively.

To estimate the mercury exposure for ospreys from the 3.03/3.04 nest site, Monte Carlo simulation was performed on the sum of the exposure estimates for each sub-reach within 5 km of the nest site. Exposure from each subreach was weighted by the proportion of the total diet it was projected to contribute. Mean (±STD) mercury exposure for ospreys from the 3.03/3.04 nest site was estimated to be 0.043±0.0041 mg/kg-d. While the 80th percentile (0.046 mg/kg-d) is less than the LOAEL (0.056 mg/kg-d), it is greater than the NOAEL (0.006 mg/kg-d). Because the LOAEL was not exceeded, adverse effects to ospreys at the 3.03/3.04 nest site are unlikely.

Effects of Retained Contaminants

To evaluate the significance of the estimated contaminant exposure and determine the nature and magnitude of potential effects, the effects of the retained COPECs must be summarized.

DDE. The 80th percentile for exposure of ospreys to DDE at subreach 3.01 exceeded the NOAEL but not the LOAEL. The osprey NOAEL and LOAEL for DDE were derived from a study of brown pelicans exposed to DDT for 5 yr (Anderson et al. 1975). Because DDE is a metabolite of DDT, effects from DDE were assumed to be comparable to those observed for DDT. Chronic exposure to 0.028 mg/kg-d DDT reduced reproductive success to 30 percent below that needed to maintain a stable population. This dose level was considered to be a LOAEL. Because an experimental NOAEL was not established, the NOAEL was estimated using LOAEL-NOAEL correction factor of 0.1. Because an experimental NOAEL was not established, the nature and exposure level at which adverse effects to individual birds may become evident cannot be defined.

Mercury. For the purposes of this assessment, it is assumed that 100 percent of the mercury (Hg) to which piscivores are exposed consists of methyl mercury, the most toxic form.

The 80th percentile for Hg exposure experienced by ospreys exceeded both the NOAEL and LOAEL at subreaches 3.01 and 3.02; exposure at all other modeled subreaches exceeded the NOAEL but not the LOAEL. Both the avian NOAEL and the LOAEL are based on a study of mallard ducks fed methyl mercury for three generations (Heinz 1979). The study was considered to represent a chronic exposure and a subchronic-chronic correction factor was not used. The only dose level administered, 0.064 mg/kg-d, caused hens to lay fewer eggs, lay more eggs outside of the nest box, and produce fewer ducklings. This dose level was considered to be a LOAEL. Because an experimental NOAEL was not established, the NOAEL was estimated using a LOAEL-NOAEL correction factor of 0.1. Based on the results of Heinz (1979), birds experiencing exposure $\geq$ LOAEL are likely to display impaired reproduction.

PCBs. The 80th percentile for PCB exposure experienced by ospreys exceeded the NOAEL but not the LOAEL at subreaches 2.04, 13, 3.01, and 3.02; a similar relationship (exposure exceeding the NOAEL but not the LOAEL) was observed for great blue heron at subreaches 2.04 and 3.02. Both the avian NOAEL and LOAEL are based on a study in which reduced egg hatchability was observed among ring-necked pheasants fed two dose levels, 1.8 and 3.6 mg/kg-d Aroclor 1254 for 17 weeks

(Dahlgren, Linder, and Carlson 1972). The study was considered to represent a chronic exposure; therefore, a subchronic-chronic correction factor was not used. Effects were observed at both dose levels; therefore, the 1.8 mg/kg-d dose level was considered to be a LOAEL. Because an experimental NOAEL was not established, the NOAEL was estimated using a LOAEL-NOAEL correction factor of 0.1. Because an experimental NOAEL was not established, the nature and exposure level at which adverse effects to individual birds may become evident cannot be defined.

Osprey Reproduction Survey

While an osprey monitoring study was not performed as part of the Clinch River Remedial Investigation, an ongoing osprey reintroduction program is being conducted by the Tennessee Wildlife Resources Agency in the Clinch/Tennessee River system. As stated previously, ospreys are nesting at three locations adjacent to the reservation: two along Melton Hill Reservoir (subreach 1) and one near K-25 on Poplar Creek approximately at the border between subreaches 3.03 and 3.04. Mean reproductive success at these three osprey nests was three young per nest (B. Anderson, personal communication). For comparison, mean reproductive success of ospreys in North American ranges from 1.7 to 2.14 young per nest (EPA 1993a).

Weight of Evidence

In this example, only two lines of evidence, literature toxicity data (consisting of comparisons of NOAELs and LOAELs to contaminant exposure estimates) and biomonitoring data (surveys of reproductive success), were available to evaluate ecological risk to ospreys. The strongest line of evidence is the biomonitoring data. Because reproductive success among the nests adjacent to the ORR is high relative to success observed among other osprey populations in North America, adverse effects are not suggested. Consideration of the literature toxicity data indicates that significant risks are present within only two subreaches (Poplar Creek 3.01 and 3.02). These risks are attributable solely to mercury. Risk from mercury is not retained, however, when exposure is recalculated taking into account the spatial component of osprey foraging behavior. Because neither line of evidence suggests that significant adverse effects are occurring to ospreys, the conclusion of the weight of evidence is that contaminants from the ORR do not present a risk to ospreys. Table 8 summarizes the weight of evidence.

Uncertainties Concerning Risks to Piscivorous Wildlife

The final step in risk characterization is to summarize the uncertainties associated with the assessment and to outline the potential impacts they may have on the

Table 8. Weight of evidence for ospreys.

Evidence	Result	Explanation
Literature Toxicity Data	-	Comparison of Hg exposure estimates to LOAELs indicates that only within two subreaches (Poplar Creek 3.01 and 3.02) are significant risks present. Risk from Hg is not retained when exposure is recalculated taking into account the spatial component of osprey foraging behavior.
Biological Surveys	-	Reproductive success of osprey adjacent to the ORR is high relative to other osprey populations in North America.
Media Toxicity Tests	NA	Toxicity tests were not performed for osprey.
Weight of Evidence	-	The weight of evidence suggests that contaminants from the ORR do not present a risk to osprey.

conclusions. The sections below describe the uncertainties associated with the previous example.

Bioavailability of contaminants. It was assumed that 100 percent of the contaminant concentration reported in fish and water was bioavailable. Much of the contaminants in biotic media are bioavailable; however, the uptake efficiencies for wildlife in the field relative to that experienced by test species is unknown. Therefore, exposure estimates based on the contaminant concentrations in media are conservative and likely to overestimate the actual contaminant exposure experienced.

Extrapolation from published toxicity data. While published toxicity studies are available for some piscivores, there are no published data for ospreys. To estimate toxicity of contaminants at the site, it was necessary to extrapolate from studies performed on test species (i.e., mallard ducks, ring-necked pheasants, etc.). While it was assumed that toxicity could be estimated as a function of body size, the accuracy of the estimate is not known. For example, ospreys may be more or less sensitive to contaminants than ducks or pheasants.

Additional extrapolation uncertainty exists for those contaminants for which data consisted of either LOAELs or were subchronic in duration. For either case, an uncertainty factor of 10 was used to estimate NOAELs or chronic data. The uncertainty factor of 10 may either over- or underestimate the actual LOAEL-NOAEL or subchronic-chronic relationship.

Toxicity of PCBs to piscivorous wildlife was evaluated using toxicity data from studies on Aroclor 1254. Because toxicity of PCB congeners can vary dramatically, the applicability of data for Aroclor 1254 is unknown.

Variable food and water consumption. While food consumption by piscivorous wildlife was assumed to be similar to that reported for the same or related species in other locations, the validity of this assumption cannot be determined. Food consumption by wildlife along the Clinch River may be greater or less than that reported in the literature, resulting in either an increase or decrease in contaminant exposure. Similarly, water consumption for all species was estimated according to the allometric equations of Calder and Braun (1983). The accuracy with which the estimated water consumption represents actual water consumption is unknown.

Single contaminant tests vs exposure to multiple contaminants in the field. While piscivores along the Clinch River are exposed to multiple contaminants concurrently, published toxicological values only consider effects experienced by exposures to single contaminants. Because some contaminants to which wildlife are exposed can interact antagonistically, single contaminant studies may overestimate their toxic potential. Similarly, for those contaminants that interact additively or synergistically, single contaminant studies may underestimate their toxic potential.

Inorganic constituents or species present in the environment. Toxicity of metal species varies dramatically depending on the valence state or form (organic or inorganic) of the metal. For example, arsenic (III) is more toxic than arsenic (V). The available data on the contaminant concentrations in media do not report which species or form of contaminant was observed. Because benchmarks used for comparison represented the more toxic species/forms of the metals, if the less toxic species/forms of the metal was actually present in fish from the Clinch River or Poplar Creek, potential toxicity at the sites may be overestimated.

Fish size selection. Data concerning the sizes of fish consumed by ospreys were obtained from the literature. Because fish sizes consumed by Clinch River piscivores may differ from that reported in the literature, exposure may be overestimated or underestimated.

10 Summary

This report identified the types of data needed to evaluate effects that smokes and obscurants have on T&E species. The effects may be evaluated using a combination of literature-based toxicity data, biological survey data, and ambient media toxicity test data. These data serve to qualitatively and quantitatively assess the relationship between contaminant exposure and direct or indirect ecological effects on T&E species.

To determine the appropriate method of data collection, this report outlined an approach that consists of identifying the T&E species of concern, identifying the contaminants of potential concern, developing a conceptual model, and selecting appropriate sampling methods based on the results of the first three steps. Three common sampling designs (random, stratified, and systematic) were also discussed.

To estimate the extent of exposure to smokes and obscurants, this report discussed methods of sampling both abiotic (water, sediment, soil, and air) and biotic (plants, birds, mammals, reptiles, amphibians, terrestrial invertebrates, benthic macroinvertebrates, and fish) media. Methods of chemical analysis of biological tissues were also covered in this report.

The report summarized available and appropriate methods of biological survey for biotic media to monitor abundance, distribution, and T&E habitat identification.

This report presents toxicity test methods for terrestrial and aquatic biota, their applications, and their strengths and weaknesses. Data generated by these methods should be used to evaluate if smokes and obscurants actually present a risk to T&E species.

Finally, the report describes an approach for estimating risks that smokes and obscurants present to T&E species based on individual lines of evidence combined through a weight-of-evidence process.

References

Cited

Addy, C.E., *Guide to waterfowl banding* (U.S. Fish and Wildlife Service, Laurel, MD, 1956).

Anderson, B.W., and R.D. Ohmart, "Vegetation," A.Y. Cooperrider, R.J. Boyd, and H.R. Stuart (eds.), *Inventory and Monitoring of Wildlife Habitat* (U.S. Department of Interior, Bureau of Land Management Service Center, Denver, CO, 1986), pp 639–60.

Anderson, D.W., R.W. Risebrough, L.A. Woods, Jr., L.R. DeWeese, and W.G. Edgecomb, "Brown pelicans: improved reproduction off the southern California coast," *Science,* vol. 190, pp 806-808 (1975).

Anderson, R.O., and S.J. Gutreuter, "Length, Weight, and Associated Structural Indices," L.A. Nielsen and D. L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 283–300.

Armour, C.L., K.P. Burnham, and W.S. Platts, *Field Methods and Statistical Analyses for monitoring Small Salmonid Streams*, FWS/OBS-83/33 (U.S. Fish and Wildlife Service, Washington, DC, 1983).

APHA (American Public Health Association), *Standard Methods for the Examination of Water and Wastewater*, 17th Ed. (Washington, DC, 1989).

ASTM (American Society for Testing and Materials), *Standard Guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing*, ASTM Standard No. E1391-90 (ASTM, Philadelphia, PA, 1990).

ASTM, *Standard Guide for Evaluation of Nematode Control Agents–Plant Responses*, ASTM Standard No. E630-91 (ASTM, 1991), pp 364–366.

ASTM, *Standard Guide for Conducting a Soil-Core Microcosm Test*, ASTM Standard No. E1197-87 (ASTM, 1993).

ASTM, *Annual Book of ASTM Standards, Water and Environmental Technology*, vol. 11.04 (ASTM, 1994).

Bagenal, T.B., and W. Nellen, "Sampling Eggs, Larvae and Juvenile Fish," T. Backiel and R.L. Welcomme, (eds.) *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 13–36.

Bailey, N.T.J., "On Estimating the Size of Mobile Populations from Recapture Data," *Biometrika* vol. 38, pp 293–306 (1951).

Bailey, N.T.J., "Improvements in the Interpretation of Recapture Data," *Journal of Animal Ecology* vol. 21, pp 120–127 (1952).

Bain, M.B., J.T. Finn, and H.E. Booke, "Quantifying Stream Substrate for Habitat Analysis Studies," *North American Journal of Fisheries Management*, vol. 5, pp 499-500 (1985).

Bain, M.B., *Evaluation of Sampling Methods and Study Designs for Quantifying Habitat Utilization by Stream Fish*, Aquatic Resources Research Series 88-1 (Alabama Cooperative Fish & Wildlife Research Unit, Auburn, AL, 1988).

Baker, A.M., and D.F. Fraser, "A Method for Securing the Gut Contents of Small, Live Fish," *Transactions of the American Fisheries Society*, vol. 1976, no. 4, pp 520–522 (1976).

Baltz, D.M., "Autecology," C.B. Schreck and P.B. Moyle (eds.), *Methods for Fish Biology* (American Fisheries Society, Bethesda, MD, 1990) pp 585–607.

Barinaga, M., "Where have all the froggies gone?" *Science*, vol. 247, pp 1033–1034 (1990).

Bartley, J.A., "Movement patterns in adult male and female mantids, *Tenodera aridifolia sinensis* Saussure (Orthoptera: Mantodea)," *Environmental Entomology*, vol. 11, pp 1108–1111 (1982).

Bates, J.M., and S.D. Dennis, *Mussel resource survey—State of Tennessee*, TWRA technical report No. 85-4 (Contract report to the Tennessee Wildlife Resources Agency, 1985).

Baudo, R., "Sediment Sampling, Mapping, and Data Analysis," R. Baudo, J. Giesy, and H. Muntau (eds.), *Sediments: Chemistry and Toxicity of In-place Pollutants*, Lewis Publ., Chelsea, MI, 1990), pp 15–60.

Baudo, R., J. Giesy, and H. Muntau, eds., *Sediments: Chemistry and Toxicity of In-place Pollutants* (Lewis Publ., Chelsea, MI, 1990).

Bayley, P.B., R.W. Larimore, and D.C. Dowling, "Electric Seine as a Fish-sampling Gear in Streams," *Transactions of the American Fisheries Society*, vol. 118, pp 447-453 (1989).

Becker, G.C., *Fishes of Wisconsin* (University of Wisconsin Press, Madison, WI, 1983).

Begon, M., *Investigating Animal Abundance: Capture-Recapture for Biologists* (University Park Press, Baltimore, 1979).

Beiswenger, R.E., "Integrating Anuran Amphibian Species into Environmental Assessment Programs," Szaro, R.C., K.E. Severson, and D.R. Patton (eds.), *Management of Amphibians, Reptiles, and Small Mammals in North America*, USDA Forest Service Gen. Tech. Rep. RM-166 (U.S. Department of Agriculture, Forest Service, 1988).

Belanger, S.E., "The Effect of Dissolved Oxygen, Sediment, and Sewage Treatment Plant Discharges upon Growth, Survival, and Density of Asiatic Clams," *Hydrobiologia*, vol. 218, pp 113–126 (1991).

Bennett, S.H., J.W. Gibbons, and J. Glanville, "Terrestrial Activity, Abundance and Diversity of Amphibians in Differently Managed Forest Types," *American Midland Naturalist*, vol. 103, pp 412–416 (1980).

Binns, N.A., "Evaluation of Habitat Quality in Wyoming Trout Streams," A. Marmelstein (ed.), *Classification, Inventory, and Analysis of Fish and Wildlife Habitat*, FWS/OBS-78/76 (U.S. Fish and Wildlife Service, Washington DC, 1977), pp 221–242.

Birchard, G.F., "Water Vapor and Oxygen Exchange in Praying Mantis (*Tenodera aridifolia sinensis*) egg cases," *Physiological Zoology*, vol. 64, pp 960–972 (1991).

Bookhout, T.A., ed., *Research and Management Techniques for Wildlife and Habits*, 5th Ed. (The Wildlife Society, Bethesda, MD, 1994).

Bowen, S.H., "Quantitative Description of the Diet," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 325-336

Brewbaker, J.L., and B.H. Kwack, "The essential role of calcium ion in pollen germination and pollen tube growth," *American Journal of Botany*, vol. 50, pp 859–865 (1963).

Brewer, L.W., C.J. Driver, R.J. Kendall, C. Zenier, and T.E. Lacher, Jr., "Effects of Methyl Parathion in Ducks and Duck Broods," *Environmental Toxicology and Chemistry*, vol. 7, pp 375–379 (1988).

Bub, H., *Bird Trapping and Bird Banding* (Cornell University Press, Ithaca, NY, 1990).

Burger, J., "Metals in Avian Feathers: Bioindicators of Environmental Pollution," *Reviews in Environmental Toxicology*, vol. 5, pp 203–311.

Burnham, K.P., D.R. Anderson, and J.L. Laake, "Estimation of Density from Line Transect Sampling of Biological Populations", *Wildlife Monograph*, vol. 72, pp 1-202 (1980).

Burris, J.A., M.S. Bamford, and A.J. Stewart, "Behavioral Responses of Marked Snails as Indicators of Water Quality," *Environmental Toxicology and Chemistry*, vol. 9, pp 69–76 (1990).

Burton, G.A., Jr., "Assessing the Toxicity of Freshwater Sediments," *Environmental Toxicology and Chemistry*, vol. 10, pp 1585–1627 (1991).

Burton, G.A., Jr., *Sediment Toxicity Assessment* (Lewis Publ., Chelsea, MI, 1992).

Bury, R.B., and P.S. Corn, "Evaluation of Pitfall Trapping in Northwestern Forests: Trap Arrays with Drift Fences," *Journal of Wildlife Management*, vol. 51, pp 112–119 (1987).

Bury, R.B., and M.G. Raphael, "Inventory Methods for Amphibians and Reptiles," J.F. Bell and T. Atterbury (eds.), *Proceedings of the International Conference. Renewable Resource Inventories for Monitoring Changes and Trends* (Oregon State University, Corvallis, OR, 1983), pp 416–419.

Calder, W.A., and E.J. Braun, "Scaling of osmotic regulation in mammals and birds", *American Journal of Physiology*, vol. 224, pp Rr601-R606 (1983).

Callahan, C.A., L.K. Russell, and S.A. Peterson, "A Comparison of Three Earthworm Bioassay Procedures for the Assessment of Environmental Samples Containing Hazardous Wastes," *Biology and Fertility of Soils*, vol. 1, pp 195–200 (1985).

Callahan, C.A., C.A. Menzie, D.E. Bumaster, D.C. Wilborn, and T. Ernst, "On-site Methods for Assessing Chemical Impact on the Soil Environment Using Earthworms: A Case Study at the Baird and McGuire Superfund Site, Holbrook, Massachusetts," *Environmental Toxicology and Chemistry*, vol. 10, pp 817–826 (1991).

Campbell, H.W., and S.P. Christman, "Field Techniques for Herpetofaunal Community Analysis," N.J. Scott, Jr. (ed.), *Herpetological Communities*, FWS Res. Rep. 13 (U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC, 1982), pp 193-200.

Carle, F.L., and M.R. Strub, "A New Method for Estimating Population Size from Removal Data," *Biometrics*, vol. 34, pp 621–630 (1978).

Cassels, D., and K. Reinbold, "Strategies for Developing a Statistically Sound Approach to Assessing Effects of Military Smokes and Obscurants on Threatened and Endangered Species," Draft Technical Report (USACERL, 1996).

Chabreck, R.H., "Methods of Capturing, Marking, and Sexing Alligators," *Proceedings of the Annual Conference of the Southeastern Association of Game and Fish Commissioners*, vol. 17, pp 47–50 (1963).

Chapman, D.G., "Some Properties of the Hypergeometric Distribution with Applications to Zoological Sample Censuses," *University of California Publications in Statistics*, vol. 1, pp 131–160 (1951).

Christie, N.D., "The Efficiency and Effectiveness of a Diver-operated Suction Sampler on a Homogenous Macrofauna," *Estuarine and Coastal Marine Science*, vol. 4, no. 6, pp 687–693 (1976).

Coker, R.E., "Freshwater Mussels and Mussel Industries of the United States," *Bulletin U.S. Bureau of Fisheries*, vol. 36, pp 13–89 (1919).

Conant, R., *A field guide to reptiles and amphibians of eastern and central North America* (Houghton Miflin Co., Boston, 1975).

Constantine, D.G., "Health Precautions for Bat Researchers," T.H. Kunz (ed.), *Ecological and Behavioral Study Methods for the Study of Bats* (Smithsonian Institution Press, Washington, D.C., 1988), pp 491-528.

Cooper, R.J., and R.C. Whitmore, "Arthropod Sampling Methods in Ornithology," *Studies in Avian Biology*, vol. 13, pp 29–37 (1990).

Cooperrider, A.Y., R.J. Boyd, and H.R. Stuart, eds., *Inventory and Monitoring of Wildlife Habitat* (U.S. Dept. Interior, Bur. Land Manage., Service Center, Denver, CO, 1986).

Corrette, B.J., "Prey Capture in the Praying Mantis *Tenodera aridifolia sinensis*: Coordination of the Capture Sequence and Strike Movements," *Journal of Experimental Biology*, vol. 148, pp 147–180 (1990).

Craig, J.F., "Sampling with Traps," T. Backiel and R.L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 55–70.

Crane, M., and L. Maltby, "The Lethal and Sublethal Responses of *Gammarus pulex* to Stress: Sensitivity and Sources of Variation in an In Situ Bioassay," *Environmental Toxicology and Chemistry*, vol. 10, pp 1331–1339 (1991).

Culp, J.M., and N.E. Glozier, "Experimental Evaluation of a Minnow Trap for Small Lotic Fish," *Hydrobiologia*, vol. 175, pp 83-87 (1989).

Dahlgren, R.B., R.L. Linder, and C.W. Carlson, "Polychlorinated biphenyls: their effects on penned pheasants", Environmental Health Perspectives, vol. 1, pp 89-101 (1972).

Dahlsten, D.L., and W.A. Copper, "The Use of Nesting Boxes to Study the Biology of the Mountain Chickadee (*Parus gambelli*) and Its Impact on Selected Forest Insects," J.G. Dickson, R.N. Conner, R.R. Fleet, J.A. Jackson, and J.C. Kroll (eds.) *The role of insectivorous birds in forest ecosystems* (Academic Press, New York, 1979), pp 217–260.

Dahm, E., "Sampling with active gear," T. Backiel and R.L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 17–89.

Davies, W.D., and W.L. Shelton, "Sampling with Toxicants," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983).

Davis, D.E, Handbook of Census Methods for Terrestrial Vertebrates (CRC Press, Boca Raton, FL, 1982a), pp199–214.

Davis, D.E. "Calculations Used in Census Methods," D.E. Davis (ed.), *Handbook of Census Methods for Terrestrial Vertebrates* (CRC Press, Inc. Boca Raton, FL, 1982b), pp 344-372.

Dawson, D.A., E.F. Stebler, S.L. Burks, and J.A. Bantle, "Evaluation of the Developmental Toxicity of Metal-contaminated Sediments Using Short-term Fathead Minnow and Frog Embryo-larval Assays," *Environmental Toxicology and Chemistry*, vol. 7, pp 27-34 (1988).

Dawson, D.A., T.WE. Schultz, and L.L. Baker, "Structure-activity Relationships for Osteolathyrism: IV. Para-substituted Benzoic Acid Hydrazides and Alkyl Carbazates," *Environmental Toxicology and Chemistry*, vol. 10, pp 455–461 (1991).

DeBrey, L.D., and J.A. Lockwood, "Effects of Sediment and Flow Regime on the Aquatic Insects of a High Mountain Stream," *Regulated Rivers: Research and Management*, vol. 5, pp 241–250 (1990).

DeGraaf, R.M., and M. Yamasaki, "A Nondestructive Technique to Monitor the Relative Abundance of Terrestrial Salamanders," *Wildlife Society Bulletin*, vol. 20, pp 260-264 (1992).

Dennis, S.D., *Distributional Analysis of the Freshwater Mussel Fauna of the Tennessee River System, with Special Reference to Possible Limiting Effects of Siltation*, TWRA No. 85-2 (Tennessee Wildlife Resources Agency, Nashville, TN, 1985).

Devillers, J., and J.M. Exbrayat (eds.), *Ecotoxicity of Chemicals to Amphibians* (Gordon and Breach Science Publ., Philadelphia, PA, 1992).

Dietrick, E.J., E.I. Schlinger, and R. Van den Bosch, "A New Method for Sampling Arthropods Using a Suction Collection Machine and a Berlese Funnel Separator, *Journal of Economic Entomology*, vol. 52, pp 1085-1091 (1959).

DOE (U.S. Department of Energy), *Remedial Investigation/Feasibility Study for the Clinch River/Poplar Creek Operable Unit*, DOE/OR/01-1393/V1&D1 (DOE, Oak Ridge, Tennessee, 1995).

DOE, *The Environmental Survey Manual*, DOE/EH-0053 (DOE, Office of Environmental Audit, Washington, DC, 1987).

Douglas, N.H., *Freshwater Fishes of Louisiana* (Claitors Publishing Division, Baton Rouge, LA, 1974).

Dumont, J.N., T.W. Schults, M.V. Buchanan, and G.L. Kao, "Frog Embryo Teratogenesis Assay: *Xenopus* (FETAX)—Short-term Assay Applicable to Complex Environmental Mixtures," M.D. Waters, S.S. Sandhu, J. Lewtas, L. Claxton, N. Chernoff, and S. Nesnow (eds.), *Short-term Bioassays in the Analysis of Complex Environmental Mixtures III* (Plenum Press, New York, NY, 1983), pp 393-405.

EPA (U.S. Environmental Protection Agency), *Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents*, EPA 670/4-73-001 (EPA, Office of Research and Development, Cincinnati, OH, 1973).

EPA, *Characterization of Hazardous Waste Sites—A Methods Manual. Vol. II. Available Sampling Methods*, EPA-600/4-83-040 (EPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV, 1983a).

EPA, *Preparation of Soil Sampling Protocol: Techniques and Strategies*, EPA-600/4-83-020 (EPA, Environmental Monitoring Systems Laboratory, Las Vegas, NV, 1983b).

EPA (U.S. Environmental Protection Agency), *Test Methods for Evaluating Solid Waste*, SW846, 3d Ed. (Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Washington, DC, 1986).

EPA, *Ecological Assessment of Hazardous Waste Sites: A Field and Laboratory Reference*, EPA/600/3-89/013 (EPA, Environmental Research Laboratory, Corvallis, OR, 1989).

EPA, *Framework for Ecological Risk Assessment. Risk Assessment Forum*, EPA/630/R-92/001 (EPA, Washington, DC, 1992a).

EPA, *Evaluation of Terrestrial Indicators for Use in Ecological Assessments at Hazardous Waste Sites*, EPA/600/R-92/183 (EPA, Office of Research and Development, Washington DC, 1992b).

EPA, *Wildlife Exposure Factors Handbook. Volume I of II*, EPA/600/R-93/187a (EPA, Office of Research and Development, Washington, DC, 1993a).

EPA, *Data Quality Objectives Process for Superfund. Interim Final Guidance*, EPA/540/G-93/071 (EPA, Washington, DC, 1993b).

EPA, "Appendix F: Recommended Procedures for Preparing Whole Fish Composite Homogenate Samples," *Guidance for Assessing Chemical Contamination for Use in Fish Advisories*, EPA/823-R-93-002 (EPA, Washington, DC, 1993c).

EPA, *Assessment and Remediation of Contaminated Sediments (ARC) Program: Assessment Guidance Document*, EPA/905-B94-002 (EPA, Great Lakes Program Office, Chicago, IL, 1994a).

EPA, *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, EPA/600/R-94/024 (Draft) (EPA, Washington, DC, 1994b).

Errington, P.L., "The Pellet Analysis Method of Raptor Food Habits Study," *Condor*, vol. 32, pp 292–296 (1930).

Etnier, D.A., and W.C. Starnes, *The Fishes of Tennessee* (The University of Tennessee Press, Knoxville, TN, 1993).

Falls, J.B., "Mapping of Territories with Playback: An Accurate Census Method for Songbirds," *Studies in Avian Biology*, vol. 6, pp 86-91 (1981).

Fenton, M.B., "Detecting, Recording, and Analyzing Vocalizations of Bats," T.H. Kunz (ed., *Ecological and Behavioral Study Methods for the Study of Bats* (Smithsonian Institution Press, Washington, DC, 1988), pp 91–104.

Ferner, J.W., *A Review of Marking Techniques for Amphibians and Reptiles*, Society for the Study of Amphibians and Reptiles, Herpetological Circular 9 (Society for the Study of Amphibians and Reptiles, 1979).

Fitch, H.S., "A Simplified Type of Funnel Trap for Reptiles," *Herpetologica*, vol.7, pp 77–80 (1951).

Fitch, H.S., "Resources of a snake community in a prairie-woodland habitat of northeastern Kansas," *in* Norman J. Scott, Jr. (ed.), *Herpetological communities*, USFWS Research Report. No. 13 (U.S. Fish and Wildlife Service, Washington, DC, 1982).

Fleet, D.R., D.R. Clark, Jr., and F.W. Plapp, Jr., "Residues of DDT and Dieldrin in Snakes from Two Texas Agro-systems," *BioScience*, vol. 22, pp 664–665 (1972).

Fletcher, J.S., F.L. Johnson, and J.C. McFarlane, "Database Assessment of Phytotoxicity Data Published on Terrestrial Vascular Plants," *Environmental Toxicology and Chemistry*, vol. 7, pp 615–622 (1988).

Forster, D.L., "A New Technique for the Daytime Capture of Adult Alligators," *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies*, vol. 45, pp 198–200 (1991).

Friend, G.R., "Relative Efficiency of Two Pitfall-drift Fence Systems for Sampling Small vertebrates," *Australian Zoology*, vol. 21, pp 423-433, (1984).

Fuller, T.K., "Wolves," D.E. Davis (ed.), *Handbook of Census Methods for Terrestrial Vertebrates* (CRC Press, Inc., Boca Raton, FL, 1982), pp 225–226.

Gale, W.F., and J.D. Thompson, "A Suction Sampler for Quantitatively Sampling Benthos on Rocky Substrates in Rivers," *Transactions of the American Fisheries Society*, vol. 104, no. 2, pp 398–405 (1975).

Galindo, J., R.J. Kendall, C.J. Driver, and T.E. Lacher, Jr., "The Effects of Methyl Parathion on Susceptibility of Bobwhite Quail to Domestic House Cat Predation," *Behavior and Neural Biology*, vol. 43, pp 21–36 (1984).

Gatz, A.J., and J.M. Loar, "Petersen and Removal Population Size Estimates: Combining Method to Adjust and Interpret Results When Assumptions Are Violated," *Environmental Biology of Fish*, vol. 21, pp 293–307 (1988).

Gavett, A.P., and J.S. Wakely, "Diets of House Sparrows in Urban and Rural Habitats," *Wilson Bulletin*, vol. 98, pp 137–144 (1986).

Gibbons, J.W., "Terrestrial Activity and the Population Dynamics of Aquatic Turtles," *American Midland Naturalist*, vol. 83, pp 404–414 (1969).

Gibbons, J.W., and D.H. Bennett, "Determination of Anuran Terrestrial Activity Patterns by a Drift Fence Method," *Copeia*, vol. 1974, no. 4, pp 236–243 (1974).

Gibbs, M.H, L.F. Wicker, and A.J. Stewart, "A Method for Assessing Sublethal Effects of Contaminants in Soils to the Earthworm, *Eisenia foetida*," *Environmental Toxicology and Chemistry* (1994, manuscript in review).

Godbout, L., and H.B.N. Hynes, "The Three Dimensional Distribution of the Fauna in a Single Riffle in a Stream in Ontario," *Hydrobiologia*, vol. 97, pp 87–96 (1982).

Goldstein, R.M., "Quantitative Comparison of Seining and Underwater Observation for Stream Fishery Surveys," *Progress in Fisheries Culture*, vol. 40, pp 108–111 (1978).

Grant, B.W., A.D. Tucker, J.E. Lovich, A.M. Mills, P.M. Dixon, and J.W. Gibbons, "The Use of Coverboards in Estimating Patterns of Reptile and Amphibian Biodiversity," D. McCulloch and R.H. Barrett (eds.), *Wildlife 2001* (Elsevier Science Publications, Inc., London, 1992), pp 379-403.

Green, R.H., *Sampling Design and Statistical Methods for Environmental Biologists* (John Wiley and Sons, Inc., New York, 1979).

Greig-Smith, P., *Quantitative Plant Ecology*, 3d Ed. (University of California Press, Berkely, CA, 1983).

Grue, C.E., G.V.N. Powell, and M.J. McChesney, "Care of Nestlings by Wild Female Starlings Exposed to an Organophosphate Pesticide," *Journal of Applied Ecology*, vol. 19, pp 327–335 (1982).

Grundel, R., and D.L. Dahlsten, "The feeding ecology of mountain chickadees (*Parus gambelli*): patterns of arthropod prey delivery to nestling birds, *Canadian Journal of Zoology*, vol. 69, pp 1793-1804 (1991).

Gullet, P.A., "Euthanasia," M. Friend (ed.), *Field Guide to Wildlife Diseases: General Field Procedures for Migratory Birds*, U.S. Fish and Wildlife Service Res. Publ. 167 (U.S. Fish and Wildlife Service, Washington, DC, 1987), pp 59–63.

Gunderson, C.A., J.M. Kostuk, M.H. Gibbs, G.E. Napolitano, L.F. Wicker, J.E. Richmond, and A.J. Stewart, "Ecological Validation of a New Remediation Technology: Does Composting TNT-contaminated Soil Make It Suitable for Land Application?" *Proceedings, 20th Environmental Symposium and Exhibition, "Department of Defense Environmental Security– Strategies for the 21st Century," March 14-17, 1994, San Antonio, Texas* (1994), pp 402–407.

Gunn, A., "Use of Mustard to Estimate Earthworm Populations," *Pedobiologia*, vol. 36, pp 65–67 (1992).

Hall, L.W., Jr., S.J. Bushong, M.C. Ziegenfuss, W.S. Hall, and R.L. Herman, "Concurrent Mobile On-site and In Situ Striped Bass Contaminant and Water Quality Studies in the Choptank River and Upper Chesapeake Bay," *Environmental Toxicology and Chemistry*, vol. 7, pp 815–830 (1988).

Hall, R.J., *Effects of Environmental Contaminants on Reptiles: A Review*, USFWS Special Scientific Wildlife Report No. 228. (U.S. Department of the Interior, U.S. Fish and Wildlife Service, Washington, DC, 1980).

Hamley, J.M., "Sampling with Gillnets," T. Backiel and R.L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 37–53.

Hankin, D.G., and G.H. Reeves, "Estimating Total Fish Abundance and Total Habitat Area in Small Streams Based on Visual Estimation Methods," *Canadian Journal of Fisheries and Aquatic Sciences*, vol. 45, pp 834–844 (1988).

Harper, J.L., *Population Biology of Plants* (Academic Press, New York, 1977).

Hartley, W.G., "The Use of Electrical Fishing for Estimating Stocks of Freshwater Fish," T. Backiel and R.L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 91–95.

Hayes, M.L., "Active Fish Capture Methods," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 123–146.

Hays, R.L., C. Summers, and W. Sietz, *Estimating wildlife habitat variables*, FWS/OBS-81/47 (U.S. Department of the Interior, Fish and Wildlife Service, Washington, DC, 1981).

Heagy, P.A., and L.B. Best, "Factors Affecting Feeding and Brooding of Brown Thrasher Nestlings," *Wilson Bulletin*, vol. 95, pp 297–303 (1983).

Heinz, G.H, "Methyl mercury: reproductive and behavioral effects on three generations of mallard ducks," *Journal of Wildlife Management*, vol. 43, pp 394-401 (1979).

Helfman, G.S., "Underwater Methods," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 349–370.

Hensler, G.L., and J.D. Nichols, "The Mayfield Method of Estimating Nesting Success: A Model, Estimators, and Simulation Results," *Wilson Bulletin*, vol. 93, pp 42–53 (1981).

Heyer, W.R., and K.A. Berven, "Species Diversity of Herpetofaunal Samples from Similar Microhabitats at Two Tropical Sites," *Ecology*, vol. 54, pp 642–645 (1973).

Heyer, W.R., M.A. Donnelly, R.W. McDiarmid, L.C. Hayek, and M.S. Foster, eds., *Measuring and Monitoring of Biological Diversity: Standard Methods for Amphibians* (Smithsonian Institution Press, Washington, DC, 1994).

Higgins, K.F., J.L. Oldemeyer, K.J. Jenkins, G.K. Clambey, and R.F. Harlow, "Vegetation Sampling and Measurement," T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats* (The Wildlife Society, Bethesda, MD, 1994), pp 567–591.

Hinzman, R.L., *Feeding-rate of a Pleurocerid Snail* (Elimia clavaeformis) *as a Laboratory Procedure for Site-specific Water Quality Assessment* (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Holden, A.V., "Chemical Methods," T. Backiel and R.L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 97–104.

Hubert, W.A., "Passive Capture Methods," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 95–122.

Hughes, R.M., and J.M. Omernik, "An Alternative for Characterizing Stream Size," T. D. Fontaine and S.M. Bartell (eds.), *Dynamics of Lotic Ecosystems* (Ann Arbor Science Publishers, Ann Arbor, MI, 1983).

Hull, R.N., and G.W. Suter II, *Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Sediment-Associated Biota: 1994 Revision*, ES/ER/TM-95/R1 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Hynes, H.B.N., *The Ecology of Running Waters* (University of Toronto Press, Toronto, 1970).

Isom, B.G., "Benthic Macroinvertebrates," W.T. Mason, Jr. (ed.), *Methods for the Assessment and Prediction of Mineral Mining Impacts on Aquatic Communities: A Review and Analysis Workshop Proceedings* (United States Fish and Wildlife Service, Harpers Ferry, WV, 1978), pp 67–74.

Jacobs, K.E., and W.D. Swink, "Estimations of Fish Population Size and Sampling Efficiency of Electrofishing and Rotenone in Two Kentucky Tailwaters," *North American Journal of Fisheries Management*, vol. 2, pp 239–248 (1982).

Jacobsen, T., and J.A. Kushlan, "Sources of Sampling Bias in Enclosure Fish Trapping: Effects on Estimates of Density and Diversity," *Fisheries Research*, vol. 5, pp 401–412 (1987).

Jenkins, R.E., and N.M. Burkhead, *Freshwater Fishes of Virginia* (American Fisheries Society, Bethesda, MD, 1993).

Jennings, M.L., H.F. Percival, and C. L. Abercrombie, "Habitat Variables Affecting Nesting Success of the American Alligator in Florida," *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies*, vol. 41, pp 334–342 (1987).

Jennings, M.L., H.F. Percival, and A.R. Woodward. "Evaluation of Alligator Hatchling and Egg Removal from Three Florida Lakes," *Proceedings of the Annual Conference of the Southeastern Association of Fish and Wildlife Agencies*, vol. 42, pp 283–294 (1988).

Johnson, C.G., and L.R. Taylor, "The Development of Large Suction Traps for Airborne Insects," *Annals of Applied Biology*, vol. 43, pp 51–62 (1955).

Johnson, D.H., "Estimating Nest Success: The Mayfield Method and an Alternative," *Auk*, vol. 96, pp 651–661 (1979).

Johnson, E.J., L.B. Best, and P.A. Heagy, "Food Sampling Biases Associated with the 'Ligature Method,'" *Condor*, vol. 82, pp 186–192 (1980).

Johnson, R.R., B.T. Brown, L.T. Haight, and J.M. Simpson, "Playback Recordings as a Special Avian Census Technique," *Studies in Avian Biology*, vol. 6, pp 68–75 (1981).

Jones, K.B., "Amphibians and Reptiles," A.Y. Cooperrider, R.J. Boyd, and H.R. Stuart (eds.), *Inventory and Monitoring of Wildlife Habitat* (U.S. Dept. Inter., Bur. Land Manage. Service Center, Denver, CO, 1986), pp 267–290.

Jolly, G.M., "Mark-recapture: What Next?" *Studies in Avian Biology*, vol. 6, pp 137–138 (1981).

Julliet, J.A., "A Comparison of Four Types of Traps Used for Capturing Flying Insects, *Canadian Journal of Zoology*, vol. 41, pp 219–223 (1963).

Kapetsky, J.M., "Addendum to Chapter 8," T. Backiel and R. L. Welcomme (eds.), *Guidelines for Sampling Fish in Inland Waters* (Food and Agricultural Organization of the United Nations, Rome, Italy, 1980), pp 105–113.

Karr, J.R., "Surveying Birds with Mist Nets," *Studies in Avian Biology*, vol. 6, pp 62–67 (1981).

Keyes, B.E., and C.E. Grue, "Capturing Birds with Mist Nets: A Review," *North American Bird Bander*, vol. 7, pp 2–14 (1982).

Koeln, G.T., L.M. Cowardin, and L.L. Strong, "Geographic Information Systems," T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, 5th Ed. (The Wildlife Society, Bethesda, MD, 1994), pp 540–566.

Krantzberg, G. "Ecosystem Health as Measured from the Molecular to the Community Level of Organization with Reference to Sediment Bioassessment," *Journal of Aquatic Ecosystem Health*, vol. 1, pp 319–328 (1992).

Krebs, C.J., *Ecological Methodology* (Harper and Row, New York, 1989).

Kszos, L.A., ed., *Report on the Biological Monitoring Program at Paducah Gaseous Diffusion Plant December 1990 to November 1992*, ORNL/TM-12338/R1 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Kunz, T.H., "Methods of Assessing the Availability of Prey to Insectivorous Bats," T.H. Kunz (ed.), *Ecological and Behavioral Methods for the Study of Bats* (Smithsonian Inst. Press, Washington, DC, 1988a), pp 191-210.

Kunz, T.H., ed., *Ecological and Behavioral Study Methods for the Study of Bats* (Smithsonian Institution Press, Washington, DC, 1988b).

Kunz, T.H., and A. Kurda, "Capture Methods and Holding Devices," T.H. Kunz (ed.), *Ecological and Behavioral Study Methods for the Study of Bats* (Smithsonian Institution Press, Washington, DC, 1988), pp 1–30.

Layher, W.G., and O.E. Maughan, "Comparison Efficiencies of Three Sampling Techniques for Estimating Fish Populations in Small Streams," *Progress in Fisheries Culture*, vol. 46, pp 180–184 (1984).

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister, and J.R. Stauffer, Jr., *Atlas of North American Freshwater Fishes*, North Carolina Biological Survey Publication 1980-12 (North Carolina State Museum of Natural History, 1980).

Licht, D.S, D.G. McCauley, J.R. Longcore, and F. Sepik, "An Improved Method To Monitor Nest Attentiveness Using Radiotelemetry," *Journal of Field Ornithology*, vol. 60, pp 251–258 (1989).

Lopez-Rojas, H., J.G. Lundberg, and E. Marsh, "Design and Operation of a Small Trawling Apparatus for Use with Dugout Canoes," *North American Journal of Fisheries Management*, vol. 4, pp 331-334 (1984).

Lowe, D.W., J.R. Matthews, and C.J. Moseley, *The Official World Wildlife Fund Guide to Endangered Species of North America, Vols. 1–3* (Beacham Publ. Inc., Washington, DC, 1994).

Luckenbach, R.A., "Ecology and Management of the Desert Tortoise, *Gopherus agassizii,* in California," Bruce Bury (ed.), *North American tortoises*, Wildlife Research Report No. 12 (Conservation and Wildlife Service, Washington DC, 1982), pp 1–38.

Lyons, J., "Capture Efficiency of a Beach-seine for Seven Freshwater Fishes in a North-temperate Lake," *North American Journal of Fisheries Management*, vol. 6, pp 288–289 (1986).

Madkour, S., and L.H. Weinstein, "Effects of Fumigation with Hydrogen Fluoride on the Loading of [^{14}C] Sucrose into the Phloem of Soybean Leaves," *Environmental Toxicology and Chemistry*, vol. 7, pp 3317–3320 (1988).

Mahon, R., "Accuracy of Catch-effort Methods for Estimating Fish Density and Biomass in Streams," *Biology of Fish*, vol. 5, pp 343–360 (1980).

Maldonado, H., K. Jaffe, and N. Balderrama, "The Dynamics of Learning in the Praying Mantis (*Stagmatoptera biocellata*)," *Journal of Insect Physiology*, vol. 25, pp 525–533 (1979).

Mallory, M.L., and P.J. Weatherhead, "A Comparison of Three Techniques for Monitoring Avian Nest Attentiveness and Weight Change," *Journal of Field Ornithology*, vol. 63, pp 428–435 (1992).

Marion, W.R., T.E. O'Meara, and D.S. Mohair, "Use of Playback Recordings in Sampling of Elusive or Secretive Birds," *Studies in Avian Biology*, vol. 6, pp 81–85 (1981).

Mattice, J.S., and W. Bosworth, "Modified Venturi Suction Sampler for Collecting Asiatic Clams," *Progess in Fisheries Culture*, vol. 41, no. 3, pp 121–123 (1979).

Mayfield, H.F. "Suggestions for Calculating Nest Success," *Wilson Bulletin*, vol. 87, pp 456-466 (1975).

Mengak, M.T., and D.C. Guynn, Jr., "Pitfalls and Snap Traps for Sampling Small Mammals and Herpetofauna," *American Midland Naturalist*, vol. 118, pp 284–288 (1987).

Meyers-Schone, L., and B.T. Walton, "Turtles as Monitors of Chemical Contaminants in the Environment," *Reviews in Environmental Contamination and Toxicology*, vol. 135, pp 93–153 (1994).

Miller, A.C., and B.S. Payne, "Qualitative Versus Quantitative Sampling To Evaluate Population and Community Characteristics at a Large-river Mussel Bed," *American Midland Naturalist*, vol. 130, pp 133–145 (1993).

Miller, A.C., B.S. Payne, D.J. Shafer, and L.T. Neill, "Techniques for Monitoring Freshwater Bivalve Communities and Populations in Large Rivers," K.S. Cummings, A.C. Buchanan, and L.M. Koch (eds.), *Conservation and Management of Freshwater Mussels* (Proceedings of an Upper Mississippi River Conservation Commission [UMRCC] symposium, 1993), pp 147–157.

Miller, H.W., and D.H. Johnson, "Interpreting Results from Nesting Studies," *Journal of Wildlife Management*, vol. 42, pp 471–476 (1978).

Mitchell, J.C., S.Y. Erdle, and J.F. Pagels, "Evaluation of Capture Techniques for Amphibian, Reptile, and Small Mammal Communities in Saturated Forested Wetlands," *Wetlands*, vol. 13, pp 30–136 (1993).

Moody, D.T., "A Method for Obtaining Food Samples from Insectivorous Birds," *Auk*, vol. 87, pp 579 (1970).

Morgan, E.L., Y.-C.A. Wu, and R.C. Young, "A Plant Toxicity Test with the Moss *Physcomitrella patens* (Hedw.) B.S.G.," W. Wang, J.W. Goresuch, and W.R. Lower (eds.), *Plants for Toxicity Assessment*, ASTM STP 1091 (ASTM, Philadelphia, PA, 1990), pp 267-279.

Morrison, D.W., R. Edmunds, G. Linscombe, and J.W. Goertz, "Evaluation of Specific Scent Station Variables in Northcentral Louisiana," *Proceedings of the Annual Conference of the Southeastern Association Fish and Wildlife Agencies*, vol. 35, pp 281–291 (1981).

Morrison, M.L., C.J. Ralph, J. Verner, and J.R. Jehl, Jr., eds., "Avian Foraging: Theory, Methodology, and Applications," *Studies in Avian Biology No. 13* (Cooper Ornithol. Soc., 1990).

Mudroch, A., and S.D. MacKnight, *Handbook of Techniques for Aquatic Sediments Sampling* (CRC Press, Boca Raton, FL, 1991).

Mueller-Dombis, D., and H. Ellenberg, *Aims and Methods of Vegetation Ecology* (John D. Wiley and Sons, New York, 1974).

Murie, O.J., *A Field Guide to Animal Tracks* (Houghton Mifflin Co., Boston, 1974).

Murkin, H.R., D.A. Wrubleski, and F.A. Ried, "Sampling Invertebrates in Aquatic and Terrestrial Habitats," T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, 5th Ed. (The Wildlife Society, Bethesda, MD, 1994), pp 349–369.

Napolitano, G.E., W.R. Hill, J.B. Guckert, A.J. Stewart, S.C. Nold, and D.C. White, "Changes in Periphyton Fatty Acid Composition in Chlorine-polluted Streams," *Journal of North American Benthological Society*, vol. 13, pp 237–249 (1993).

Newell, P.F., "Mollusks: Methods for Estimating Production and Energy Flow," J. Phillipson (ed.), *Methods of Study in Soil Ecology* (United Nations Educational, Scientific, and Cultural Organization [UNESCO], 1970), pp 285–291.

Nichols, J.D., B.R. Noon, S.L. Stokes, and J.E. Hines, "Remarks on the Use of Mark-recapture Methodology in Estimating Avian Population Size," *Studies in Avian Biology*, vol. 6, pp 121–136 (1981).

Oelke, H., "Limitations of the Mapping Method," *Studies in Avian Biology*, vol. 6, pp 114–118 (1981).

Opresko, D.M., B.E. Sample, and G.W. Suter II, *Toxicological Benchmarks for Wildlife: 1994 Revision*, ES/ER/TM-86/R1 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Orth, D.J., "Aquatic Habitat Measurements," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983).

Otis, D.L., K.P. Burnham, G.C. White, and D.R. Anderson, "Statistical Inference from Capture Data on Closed Animal Populations," *Wildlife Monograph*, vol. 62, pp 1–135 (1978).

Parmalee, R.W., R.W. Wentsel, C.T. Phillips, M. Simini, and R. Checkai, "Soil Microcosm for Testing the Effects of Chemical Pollutants on Soil Fauna Communities and Trophic Structure," *Environmental Toxicology and Chemistry*, vol. 12, pp 1477–1486 (1993).

Perrins, C.M., "Population Fluctuations and Clutch Size in the Great Tit, *Parus major* L.," *Journal of Animal Ecology*, vol. 34, pp 601–647 (1965).

Petersen, R.C., Jr., "The RCE: A Riparian, Channel, and Environmental Inventory for Small Streams in the Agricultural Landscape, *Freshwater Biology*, vol. 27, pp 295–306 (1992).

Peterson, M.J., and G.R. Southworth, "Bioaccumulation Studies. PCBs in Caged Clams," J.G. Smith (ed.), *Second Report on the Oak Ridge K-25 Site Biological Monitoring and Abatement Program for Mitchell Branch*, Environmental Sciences Division Publication No. 3928 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Peterson, N.P., and C.J. Cederholm, "A Comparison of the Removal and Mark-recapture Methods of Population Estimation for Juvenile Coho Salmon in a Small Stream," *North American Journal of Fisheries Management*, vol. 4, pp 99–102 (1984).

Pianka, E.R., *Evolutionary Ecology* (Harper and Row, New York, NY, 1974).

Platts, W.S., W.F. Megahan, and G.W. Minshall, *Methods for Evaluating Stream, Riparian, and Biotic Conditions*, USFS Report INT-138 (U.S. Dept. Agriculture, Forest Service, Ogden, Utah, 1983)

Platts, W.S., C. Armour, G.D. Booth, M. Bryant, and J.L. Bufford, *Methods for Evaluating Riparian Habitats with Applications to Management*, USFS Report INT-221 (U.S Dept. Agriculture, Forest Service, Ogden, Utah, 1987).

Policastro, A.J., D.M. Maloney, W.E. Dunn, J.C. Liljegren, and G.E. DeVaull, *Field Measurement and Model Evaluation Program for Assessment of the Environmental Effects of Military Smokes*, NTIS/AD-A216 054/7 (1990).

Pontasch, K.W., and M.A. Brusven, "Diversity and Community Comparison Indices: Assessing Macroinvertebrate Recovery Following a Gasoline Spill," *Water Research*, vol. 22, no. 5, pp 629–626 (1988).

Pontasch, K.W., and M.A. Brusven, "Macroinvertebrate and Periphyton Response to Streambed Agitation for Release of Substrate-trapped Hydrocarbons," *Archives of Environmental Contamination and Toxicology*, vol. 18, pp 545-553 (1989).

Price, T. "Diet Variation in a Population of Darwin's Finches," *Ecology*, vol. 68, pp 1015–1028 (1987).

Ralph, C.J., and J.M. Scott, eds., "Estimating Numbers of Terrestrial Birds," *Studies in Avian Biology. No. 6* (Cooper Ornithol. Soc., 1981).

Ralph, C.P., S.E. Nagata, and C.J. Ralph, "Analysis of Droppings To Describe Diets of Small Birds," *Journal of Field Ornithology*, vol. 56, pp 165–174 (1985).

Rand, G.M., and S.R. Petrocelli, *Fundamentals of Aquatic Toxicology* (Hemisphere Publishing Corp., Washington, DC, 1985).

Rankin, E.T., *The Qualitative Habitat Evaluation Index [QHEI]: Rationale, Methods, and Application* (Ohio Environmental Protection Agency, Columbus, OH, 1989).

Rattner, B.A., and J.C. Franson, "Methyl Parathion and Fenvalerate Toxicity in American Kestrels: Acute Physiological Responses and Effects of Cold," *Canadian Journal of Physiological Pharmacology*, vol. 62, pp 787–792 (1983).

Raw, F. "Estimating Earthworm Populations Using Formalin," *Nature*, vol. 148, pp 1661–1662 (1959).

Reynolds, J.B., "Electrofishing," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 147–164.

Reynolds, R.P. and N.J. Scott, Jr., "Use of a Mammalian Resource by a Chihuahuan Snake Community," Norman J. Scott Jr. (ed.) *Herpetological Communities*, USFWS Research Report No. 13 (U.S. Fish and Wildlife Service, Washington, DC, 1982).

Reynolds, R.T., J.M. Scott, and R.A. Nussbaum, "A Variable Circular-plot Method for Estimating Bird Numbers," *Condor*, vol. 82, pp 309–313 (1980).

Riley, S.C., and K.D. Fausch, "Underestimation of Trout Population Size by Maximum-likelihood Removal Estimates in Small Streams," *North American Journal of Fisheries Management*, vol. 12, pp 768–776 (1992).

Riley, S.C., R.L. Haedrich, and R.J. Gibson, "Negative Bias in Removal Estimates of Atlantic Salmon Parr Relative to Stream Size," *Freshwater Ecology*, vol. 8, pp 97–101 (1993).

Robinson, S.K., and R.T. Holmes, "Foraging Behavior of Forest Birds: The Relationships Among Search Tactics, Diet, and Habitat Structure," *Ecology*, vol. 63, pp 1918–1931 (1982).

Robison, H.W., and T.M. Buchanan, *Fishes of Arkansas* (University of Arkansas Press, Fayetteville, AK, 1988).

Rosenberg, K.V., and R.J. Cooper, "Approaches to Avian Diet Analysis," *Studies in Avian Biology*, vol. 13, pp 80–90 (1990).

Rossel, S., "Binocular Spatial Localization in the Praying Mantis," *Journal of Experimental Biology*, vol. 120, pp 265–281 (1986).

Rotenberry, J.T., "Dietary Relationships Among Shrubsteppe Passerine Birds: Competition or Opportunism in a Variable Environment?" *Ecological Monograph*, vol. 50, pp 93–110 (1980).

Royama, T., "A Device of an Auto-cinematic Food Recorder," *Tori*, vol. 15, pp 172–176 (1959).

Royama, T., "Factors Governing the Hunting Behavior and Selection of Food by the Great Tit, *Parus major*," *Journal of Animal Ecology*, vol. 39, pp 619–660 (1970).

Ryder, R.A., "Songbirds," A.Y. Cooperrider, R.J. Boyd, and H.R. Stuart (eds.), *Inventory and Monitoring of Wildlife Habitat* (U.S. Dept. Interior, Bureau of Land Management, Service Center, Denver, CO, 1986), pp 291–312.

Sample, B.E., and G.W. Suter II, "Estimating Exposure of Terrestrial Wildlife to Contaminants," ES/ER/TM-125 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Samuel, M.D., and M.R. Fuller, "Wildlife Radiotelemetry," T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, 5th Ed. (The Wildlife Society, Bethesda, MD, 1994).

Sasson-Brickson, G., and G.A. Burton, "In Situ and Laboratory Sediment Toxicity Testing with *Ceriodaphnia dubia*," *Environmental Toxicology and Chemistry*, vol. 10, pp 201–207 (1991).

Satchell, J.E. "Measuring Population and Energy Flow in Earthworms," J. Phillipson (ed.) *Methods of Study in Soil Ecology* (UNESCO, 1970), pp 261–267.

Schaeffer, D.J., E.W. Novak, W.R. Lower, A. Yanders, S. Kapila, and R. Wang, "Effects of Chemical Smokes on Flora and Fauna under Field and Laboratory Exposures," *Ecotoxicology and Environmental Safety*, vol. 13, pp 301–315 (1987).

Schemnitz, S.D., "Capturing and Handling Wild Animals," T.A. Bookhout (ed.), *Research and Management Techniques for Wildlife and Habitats*, 5th Ed. (The Wildlife Society, Bethesda, MD, 1994) pp 106–124.

Seber, G.A.F., and E.D. LeCren, "Estimating Population Parameters from Catches Large Relative to the Population," *Journal of Animal Ecology*, vol. 36, pp 631–643 (1967).

Sherry, T.W., "Comparative Dietary Ecology of Sympatric Insectivorous Neotropical Flycatchers (Tyrannidae)," *Ecological Monograph*, vol. 54 pp 313–338 (1984).

Shine, R. *Diets and abundances of aquatic and semi-aquatic reptiles in the Alligator Rivers region*. Technical Memorandum No. 16. (Australian Government Publishing Service, Canberra, Australia, 1986).

Sidle, J.G., "Critical Habitat Designation: Is It Prudent?" *Environmental Management*, vol. 11, pp 429–437 (1987).

Smith, J.G., "Instream Ecological Monitoring: Benthic Macroinvertebrates," J.G. Smith (ed.), *Second Report on the Oak Ridge K-25 Site Biological Monitoring and Abatement Program for Mitchell*

Branch, Environmental Sciences Division Publication No. 3928 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Smith, W.P., D.J. Twedt, D.A. Wiedenfeld, P.B. Hamel, R.P. Ford, and R.J. Cooper, *Point Counts of Birds in Bottomland Hardwood Forests of the Mississippi Alluvial Valley: Duration, Minimum Sample Size, and Points Versus Visits*, Res. Paper SO-274 (U.S. Dept. Agri. Forest Service, SE Forest Exp. Sta., New Orleans, LA, 1993).

Smith-Vaniz, W.F., *Freshwater Fishes of Alabama*, Paragon Press, Montgomery, AL, 1968.

Smock, L.A., J.E. Gladden, J.L. Riekenberg, L.C. Smith, and C.R. Black, "Lotic Macroinvertebrate Production in Three Dimensions: Channel Surface, Hyporheic, and Floodplain Environments," *Ecology*, vol. 73, no. 3, pp 876–886 (1992).

Snyder, D.E., "Fish Eggs and Larvae," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 165–198.

Southwood, T.R.E., *Ecological Methods* (Chapman and Hall, New York, 1978).

Southworth, G.R., and M.J. Peterson, "Bioaccumulation Studies," R.L. Hinzman (ed.), *Second Report on the Oak Ridge Y-12 Plant Biological Monitoring and Abatement Program of East Fork Poplar Creek*, Y/TS-888 (Oak Ridge National Laboratory, Oak Ridge, TN, 1993).

Spellerberg, I.F., *Biology of Reptiles: An Ecological Approach* (Blackie and Son Ltd., London, 1982).

Spillers, D.M., and D.W. Speake, "A Survey Method for Measuring Gopher Tortoise Density and Habitat Distribution," R.C. Szaro, K.E. Severson, and D.R. Patton (eds.), *Management of Amphibians, Reptiles, and Small Mammals in North America*," USDAFS Gen. Tech. Rep. RM-166 (U.S. Department of Agriculture, Forest Service, 1988), pp 199–204.

Springett, J.A., "A New Method for Extracting Earthworms from Soil Cores, with a Comparison of Four Commonly Used Methods for Estimating Populations," *Pedobiologia*, vol. 21, pp 217–222 (1981).

Starrett, W.C., "A Survey of the Mussels (Unionacea) of the Illinois River: A Polluted Stream," *Illinois Natural History Survey Bulletin*, vol. 30, no. 5, pp 267–403 (1971).

Stebbins, R.C., *A Field Guide to Western Reptiles and Amphibians* (Houghton Mifflin Co., Boston, 1966).

Stewart, A.J., "Toxicity Monitoring," J.M. Loar (ed.), *Third Report on the Oak Ridge National Laboratory Biological Monitoring and Abatement Program for White Oak Creek Watershed and the Clinch River*, ORNL/TM-11358 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Stewart, A.J., W.R. Hill, K.D. Ham, S.W. Christensen, and J.J. Beauchamp, "Chlorine dynamics and toxicity in receiving streams," *Ecological Applications*, in press (1994).

Stickney, R.R., "Care and Handling of Live Fish," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 85–94.

Strommer, J.L., and L.A. Smock, "Vertical Distribution and Abundance of Invertebrates Within the Sandy Substrate of a Low-gradient Headwater Stream," *Freshwater Biology*, vol. 22, pp 263–274 (1989).

Summerfelt, R.C., and L.S. Smith, "Anesthesia, Surgery, and Related Techniques," C.B. Schreck and P.B. Moyle (eds.), *Methods for Fish Biology* (American Fisheries Society, Bethesda, MD, 1990), pp 213–272.

Suter, G.W., II, ed., *Ecological Risk Assessment* (Lewis Publishers, Chelsea, MI, 1993).

Suter, G.W., II, and J.B. Mabrey, *Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Aquatic Biota: 1994 Revision*, ES/ER/TM-96/R1 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994).

Swenson, J.E., "Ospreys," D.E. Davis (ed.), *Handbook of Census Methods for Terrestrial Vertebrates* (CRC Press, Boca Raton, FL, 1982), pp 55–56.

Szaro, R.C., S.C. Belfit, J.K. Aitkin, and R.D. Babb, "The Use of Timed Fixed-area Plots and a Mark-recapture Technique in Assessing Riparian Garter Snake Populations," R.C. Szaro, K.E. Severson, and D.R. Patton (eds.), *Management of Amphibians, Reptiles, and Small Mammals in North America*, USDAFS Gen. Tech. Rep. RM-166 (U.S. Department of Agriculture, Forest Service, Washington, DC, 1988), pp 239–246.

Temple, P.J., and R. Wills, "Sampling and Analysis of Plants and Soil,"W.W. Heck, S.V. Krupa, and S.N. Linzon (eds.), *Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation* (1979), pp 13-1 to 13-23.

Terrell, J.W., T.E. McMahon, P.D. Inskip, R.F. Raleigh, and K.L. Williamson, "Appendix A," Guidelines for Riverine and Lacustrine Applications of Fish HSI Models With the Habitat Evaluation Procedures," *Habitat Suitability Index Models*, FWS/OBS-82.10A (U.S. Fish Wildlife Service, Washington DC, 1982).

Theimer, T.C., and G.C. Bateman, "Patterns of Prickly-pear Herbivory by Collared Peccaries," *Journal of Wildlife Management*, vol. 56, pp 234–240 (1992).

Thomas, D.W., and R.K. LaVal, "Survey and Census Methods," T.H. Kunz (ed.), *Ecological and Behavioral Study Methods for the Study of Bats* (Smithsonian Institution Press, Washington, DC, 1988).

Thompson, R.L., "Red-cockaded woodpecker," D.E. Davis (ed.), *Handbook of Census Methods for Terrestrial Vertebrates* (CRC Press, Boca Raton, FL, 1982), pp 91–92.

Trautman, M.B., *The Fishes of Ohio*, (Ohio State University Press, Columbus, OH, 1981).

U.S. Fish and Wildlife Service and Canadian Wildlife Service, *North American Bird Banding Manual. Vol. II* (U.S. Dept. of Interior, Washington, DC, 1977).

Valiente, B.A., and E. Ezxurra, "Shade as a Cause of the Association Between the Cactus *Neobuxbaumia tetetzo* and the Nurse Plant *Mimosa lluisana* in the Tehuacan Valley, Mexico," *Journal of Ecology*, vol, 79, pp 961–971 (1991).

Van Daele, L.J., and H.A. Van Daele, " Factors affecting the productivity of ospreys nesting in west-central Idaho," *Condor*, vol. 84, pp 292-299 (1982).

Varney, J.R., and D.J. Ellis, "Telemetering Egg for Use in Incubation and Nesting Studies," *Journal of Wildlife Management*, vol. 38, pp 142–148 (1974).

Verner, J., "Assessment of Counting Techniques," *Current Ornithology*, vol. 2, pp 247–302 (1985).

Verollet, P.G., and H. Tachet, "A Suction Sampler for Sampling Benthic Macroinvertebrates in Large Rivers," *Archives of Hydrobiology*, vol. 84, pp 55–64 (1978).

Vogt, R.C., and R.L. Hine, "Evaluation of Techniques for Assessment of Amphibian and Reptile Populations in Wisconsin," N.J. Scott, Jr. (ed.), *Herpetological Communities*, USFWS Wildl. Res. Rep. 13 (U.S. Department of the Interior, U.S. Fish and Wildlife Service, 1982), pp 201–217.

Wakely, J.S., "Avian Territory Mapping," (Sect. 6.3.4) *U.S. Army Corps of Engineers Wildlife Resources Management Manual*, Tech. Rept. EL-87-7 (U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 1987a).

Wakely, J.S., "Avian Line-transect Methods," (Sect. 6.3.2) *U.S. Army Corps of Engineers Wildlife Resources Management Manual*, Tech. Rept. EL-87-5 (U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 1987b).

Wakely, J.S., "Avian Plot Methods," (Sect. 6.3.3) *U.S. Army Corps of Engineers Wildlife Resources Management Manual*, Tech. Rept. EL-87-6 (U.S. Army Engineer Waterways Experiment Station, Vicksburg, MS, 1987c).

Weber, C.I., W.H. Peltier, T.J. Norberg-King, W.B. Horning, F.A. Kessler, J.R. Menkedick, T.W. Neiheisel, P.A. Lewis, D.J. Klemm, Q.H. Pickering, E.L. Robinson, J.M. Lazorchak, L.J. Wymer, and R.W. Freyberg, *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA/600/4-89/00 (EPA, Cincinnati, OH, 1989).

Weber, C.I., (ed.), *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, EPA/600/4-90/027F, 4th Ed. (EPA, Cincinnati, OH, 1993).

Wentsel, R.S., R.T. Checkai, T. W. LaPoint, M. Simini, D. Ludwig, and L. Brewer, *Procedural Guidelines for Ecological Risk assessment at U.S. Army Sites, Volume I*, ERDEC-TR-221 (Edgewood Research, Development and Engineering Center, Aberdeen Proving Ground, MD, 1994).

Whaley, M., R. Garcia, and J. Sy, "Acute Bioassays with Benthic Macroinvertebrates Conducted in Situ," *Bulletin of Environmental Contamination and Toxicology*, vol. 43, pp 570–575 (1989).

White, D.H., G.A. Mitchell, and E.F. Hill, "Parathion Alters Incubation Behavior of Laughing Gulls," *Bulletin of Environmental Contamination and Toxicology*, vol. 23, pp 281–284 (1983).

White, G.C., D.R. Anderson, K.P. Burnham, and D.L. Otis, *Capture-recapture and Removal Methods for Sampling Closed Populations*, LA-8787NERP (Los Alamos National Laboratory, Los Alamos, NM, 1982).

Whitmore, R.C., "Grasshopper Sparrow," Davis, D.E. (ed.), *Handbook of Census Methods for Terrestrial Vertebrates* (CRC Press, Boca Raton, FL, 1982), p. 112.

Wiley, M.L., and C-F. Tsai, "The Relative Efficiencies of Electrofishing vs. Seines in Piedmont Streams of Maryland," *North American Journal of Fisheries Management*, vol. 3, pp 243–253 (1983).

Will, M.E., and G.W. Suter II, *Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Terrestrial Plants*, ES/ER/TM-85/R1 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994a).

Will, M.E., and G.W. Suter II, *Toxicological Benchmarks for Screening Potential Contaminants of Concern for Effects on Soil and Litter Invertebrates and Heterotrophic Processes*, ES/ER/TM-126 (Oak Ridge National Laboratory, Oak Ridge, TN, 1994b).

Williams, D.D., and H.B.N. Hynes, "The Occurrence of Benthos Deep in the Substratum of a Stream," *Freshwater Biology*, vol. 4, pp 233–256 (1973).

Winterstein, S.R., "Chi-square Tests for Intrabrood Independence When Using the Mayfield Method," *Journal of Wildlife Management*, vol. 56, pp 398–402 (1992).

Wydoski, R., and L. Emery, "Tagging and Marking," L.A. Nielsen and D.L. Johnson (eds.), *Fisheries Techniques* (American Fisheries Society, Bethesda, MD, 1983), pp 215–238.

Young, H.J., and M.L. Stranton, "Influence of Environmental Quality of Pollen Competitive Ability in Wild Radish," *Science*, vol. 248, pp 1631–1633 (1990).

Zach, R., and J.B. Falls, "Bias and Mortality in the Use of Tartar Emetic to Determine the Diet of Ovenbirds (Aves:Parulidae)," *Canadian Journal of Zoology*, vol. 54, pp 1599–1603 (1976).

Ziegenfuss, M.C., L.W. Hall, Jr., S.J. Bushong, J.A. Sullivan, and M.A. Unger, "A Remote In Situ Apparatus for Ambient Toxicity Testing of Larval and Yearling Fish in River or Estuarine Systems," *Environmental Toxicology and Chemistry*, vol. 9, pp 1311–1315 (1990).

Zippin, C., "An Evaluation of the Removal Method of Estimating Animal Populations," *Biometrics*, vol. 12, pp 163–169 (1956).

Zippin, C., "The Removal Method of Population Estimation," *Journal of Wildlife Management*, vol. 22, pp 82–90 (1958).

Uncited

EPA, *Wildlife criteria portions of the proposed water quality guidance for the Great Lakes System,* EPA/822/R-93/006 (EPA, Office of Science and Technology, Washington, DC, 1993d).

Morales, L.M.R., and M. Goutx, "Effects of Water-soluble Fraction of the Mexican Crude Oil 'Isthmus Cactus' on Growth, Cellular Content of Chlorophyll a, and Lipid Composition of Planktonic Microalgae," *Marine Biology*, vol. 104, pp 503–509 (1990).

Abbreviations and Acronyms

AC	alternating current
APHA	American Public Health Association
ARAR	applicable or relevant and appropriate requirement
ASTM	American Society for Testing and Materials
CAM	Crassulacean acid metabolism
CDC	Centers for Disease Control
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CLP	Contract Laboratory Program
COPC	contaminant of potential concern
COPEC	contaminant of potential ecological concern
DC	direct current
DOE	U.S. Department of Energy
DQO	Data Quality Objective
EPA	U.S. Environmental Protection Agency
ERA	Ecological Risk Assessment
FDA	Food and Drug Administration
GIS	Geographic Information System
GPS	Global Positioning System
HQ	hazard quotient
HQI	Habitat Quality Index
LOAEL	lowest observed adverse effects level
NOAEL	no observed adverse effects level
QC	Quality Control
QHEI	Qualitative Habitat Evaluation Index
SOP	standard operating procedure
T&E	threatened and endangered
TN	trap night
TU	toxic unit
USACERL	U.S. Army Construction Engineering Research Laboratories
USFWS	U.S. Fish and Wildlife Service
UV-B	Ultraviolet-B
WWF	World Wildlife Fund

Appendix A: Standard Operating Procedures for Collection of Benthic Macroinvertebrates

BENTHIC MACROINVERTEBRATE MONITORING SAMPLE COLLECTION AND STORAGE

SECTION SOP-5
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DATE 10-31-91

BMAP QUALITY ASSURANCE PLAN

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

Purpose

Provide procedures for the collection of quantitative benthic macroinvertebrate samples from streams and White Oak Lake.

Equipment

95% denatured ethyl alcohol
16 oz polyurethane coated glass jars
1" X 1 1/2" self-adhesive labels
3/4" X 1" inner sample labels
"Radioactive Material" labels
NFPA chemical identification labels
Surber square foot stream bottom sampler (368-μm mesh net)
Hess stream bottom sampler (368-μm mesh net)
Ponar grab sampler with a two meter piece of attached heavy duty rope
8" X 10" plastic white photo trays (2)
Squirt bottle
Forceps
Meter stick
Rubber overshoes or hip boots
Shoulder length black neoprene-coated gloves
Disposable latex laboratory gloves
Yellow C-area coveralls
Soft bristled brush
Horiba Model U-7 Water Quality Checker
Sample field data sheets (Exhibits 5-1 and 5-2)
Benthic macroinvertebrate sample chain-of-custody forms (Exhibit 7-1)
Clipboard or aluminum carrying case for field data sheets
No. 2 lead pencil
Pen with waterproof ink
Large yellow plastic bags (four)
Box for storing samples during transport (see SOP-8)
Portable radiation survey meter (beta/gamma)

Procedures

1. Following the steps in SOP-4, select sampling locations for each site to be sampled. If samples are collected from a "Regulated Area" (i.e., White Oak Lake, lower White Oak Creek, [illegible] Melton Branch), personnel shall wear appropriate protective clothing. Each person shall wear yellow C-area coveralls; the person collecting the samples shall wear shoulder length neoprene

gloves and the person processing the sample shall wear disposable latex laboratory gloves. Before reentering a vehicle and proceeding elsewhere; all personnel shall scan themselves and their equipment, and handle contaminated materials per procedures in QA-BMAP-19-200.

2. **Collection of Benthic Macroinvertebrate Samples from Streams**

a. The type of sampling device used and the number of replicates to be collected are specified in each monitoring plan. Stream samples are collected with either a Surber square foot stream bottom sampler or a Hess stream bottom sampler. If additional sampling sites are added to a monitoring program at a later date, the same sampling device used for this program will be used at the new site as well.

b. Prior to collection of the first invertebrate sample, measure selected water quality parameters with the Horiba meter following the procedures described in SOP-3; water quality parameters are measured only once at each site on each collection date. Enter the data in the appropriate columns on the sample field data sheet (Exhibits 5-1 and 5-2).

c. On the sample field data sheet (Exhibit 5-1), enter the date, beginning time, site name, type of sample being collected (N = quantitative sample), the initials of the person collecting the sample, and the initials of the person recording the data and placing the sample in the sample jar. (See Exhibit 5-2 for instructions for entering data on the field data sheet).

d. Don shoulder length black-neoprene gloves and enter the stream with the sampler and a meter stick just downstream of the study riffle. Beginning at the bottom of the riffle, move upstream and stop one transect downstream of the first transect to be sampled. Each transect is approximately equal to one normal adult step or a distance of about one meter. Moving parallel to the transect (across stream), stop at the cell located directly downstream of the cell to be sampled. While leaning forward over the cell to be sampled or while kneeling on one knee, securely place the sampling device on the bottom of the stream within the cell to be sampled with the open end of the collection net facing upstream and the tip of the collection net pointing downstream. Make sure that the frame of the sampling device is placed securely on the bottom of the stream, and that a good seal is obtained between the frame and the substrate. If necessary, carefully shift the frame around in the immediate vicinity of the cell while holding the sampler just off of the bottom of the stream, avoiding disturbing the substrate while doing this.

e. Obtain replicate-specific characteristics following the procedures in SOP-2.

f. Next, pick up large rocks or other large debris within the frame of the sampling device, and while holding each large piece of material just under the surface of the water in the mouth of the net, rub off any clinging organisms and/or attached structures built by organisms (e.g., rock cases of caddisflies) by hand and/or with a soft bristled brush and

allow them to drift into the collection net. After rubbing/brushing the rocks, visually inspect the rock/piece of debris to insure that all organisms and their cases have been removed. Then place the rocks/debris to either side or just downstream of the sampling device but never upstream of the sampling device. Rocks partially inside the sample area should be included only if greater than half is within the sample area.

g. After all large rocks and debris have been cleaned and removed, gently but thoroughly disturb, by hand, the finer substratum within the frame of the sampling device to a depth of approximately 5 cm to 10 cm. This is accomplished by repeatedly digging, stirring, and swirling the substrate, which dislodges the invertebrates and allows the current to carry them into the collection net. Repeat this step twice, allowing the water to clear within the sample area between each repetition. Care should be taken to include the entire area within the frame of the sampling device. The edges of the sample area can be sampled effectively by pushing sediment away from the edges to the middle of the sample area and then repeating the process of disturbing the finer substratum as described above.

h. After the sampling area has been thoroughly disturbed to dislodge the organisms and the disturbed material has washed into the collection net, tilt the sampling device backwards approximately 45°, and then quickly and forcefully lift it from the water to allow the sample debris to be washed to the tip of the collection net. Being careful to avoid submerging the mouth of the net, dip the net back into the water and again rapidly and forcefully pull it completely out of the water. Repeat this procedure several times until the sample debris is concentrated into the tip of the collection net. The contents of the net may also be washed to the tip by splashing water on the outside of the net above any clinging debris.

i. Next, transfer the sample debris to a 16-oz, polyurethane-coated glass jar. Samples collected with the Surber sampler may be transferred by either direct transferal (step j below) or by transferring the sample debris to a photo tray (step l below). Samples collected with the Hess sampler are transferred directly (step l below). Prior to transfer, place a label inside the sample jar **and** a self-adhesive label on the outside top of the jar lid with the following information on each: site name, sample number, date, and chain-of-custody number (see SOP-7; step 1a). Additionally, place an appropriately marked NFPA self-adhesive identification label on each jar; appropriate markings include chemical name (ethanol) and the health, flammability, and reactivity numbers (1, 4, and 0, respectively, for ethanol). If the sample has been collected from an area potentially contaminated with radionuclides (see SOP-9), place a self-adhesive "Radioactive Material" label on the exterior side of the jar.

j. Direct Transfer - Surber Samples

(1) Fill a sample jar approximately one-third to one-half full of 95% denatured alcohol.

(2) After the debris from the sample has been washed to the tip of the net, grab the tip of the net and its contents and invert the net while maintaining a grasp around the sample contents. Next, carefully empty the sample contents into the jar while holding the jar over an 8" X 10" white photo tray. Use of the tray avoids loss of any spillage.

(3) Reinvert the net and again wash the remaining contents to the tip of the collection net following the procedures in step 2h above.

(4) Repeat this process until all of the sample debris in the net has been transferred to a sample jar.

(5) Carefully turn the sample net inside out and examine it for clinging organisms. Any organisms found should be carefully removed with forceps and placed into the sample jar.

k. Transfer by Tray - Surber Samples

(1) After washing the sample debris to the tip of the collection net, grab the outside tip of the net with its contents, invert the net while still grasping the tip, and empty the contents into an 8" X 10" white plastic photo tray containing a small amount of 95% denatured ethanol.

(2) Gently swirl the alcohol in the tray to concentrate the sample debris into one corner. While holding a sample jar over another 8" X 10" white plastic photo tray, pour the contents into the jar.

(3) Reinvert the collection net and wash the remaining contents back into the tip of the net by following step h above.

(4) Repeat the process of emptying the sample debris into the tray of alcohol and sample jar. This process should be repeated until all of the sample debris has been transferred from the tray and net into the jar.

(5) Carefully turn the collection net inside out and examine it for clinging organisms. Any organisms found should be carefully removed with forceps and placed into the sample jar.

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

(6) If a sample jar is overfilled with alcohol, carefully pour approximately half of the liquid from the jar and all of the liquid from the overflow tray back through the Surber net. Repeat steps (2) - (5) above for transferring a sample from the collection net and tray to a sample jar.

L. **Transfer of Hess Samples**

(1) Carefully remove the sample bucket from the net and rinse the inside walls with 95% denatured ethanol using a squirt bottle to force the contents to the bottom of the bucket, leaving some liquid in the bottom of the bucket.

(2) While holding the sample jar over an 8" X 10" plastic white photo tray, carefully pour the contents of the bucket into a sample jar.

(3) Again, wash the material remaining on the inside walls and screen to the bottom of the bucket with ethanol. This process should be repeated until all sample material on the inside of the bucket has been washed into the jar.

(4) Examine the mesh on the side of the sample bucket and place any clinging organisms in the sample jar with forceps.

(5) Finally, carefully turn the net of the Hess sampler inside out and place any clinging organisms into the sample jar with forceps.

(6) If a sample jar is overfilled with alcohol carefully pour approximately half of the liquid from the jar and all of the liquid from the overflow tray back through the Hess bucket. Then repeat steps (1) - (4) above for transferring a sample from the sample bucket to a sample jar.

3. After taking the last sample at a site, invert the net of the sampling device and thoroughly rinse it out in the stream. For the Hess sampler, rinse both the net and bucket.

4. Place each sample jar into a shipping container for transport following the procedures in SOP-8.

5. Within one week after collection, replace the alcohol of each sample jar, and dispose of any liquid and solid low-level compactible wastes following the procedures in SOP-9.

6. At the end of each day, enter a summary of the day's sampling activities in a registered logbook maintained in the PI's office (Building 1905, Room 308). Additional information should also be included in the summary such as weather, unusual conditions, any problems encountered, etc.

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

7. **Collection of Benthic Macroinvertebrate Samples from White Oak Lake**

   a. Follow procedures in step 1 of SOP-5 prior to and after completing work in White Oak Lake.

   b. Prior to collecting the first sample, obtain selected water quality measurements from the surface of White Oak Lake with an Horiba U-7 Water Quality Checker following the procedures in SOP-3; obtain only one measurement for each parameter on each collection date.

   c. Quantitative samples are collected in White Oak Lake from a boat with a Petite Ponar grab sampler (15 cm X 15 cm). Five samples are taken from a permanently marked transect (see SOP-1). The first sampling cell is located approximately 10 m (two boat lengths) from the north bank (right bank facing the dam). Take the remaining four samples at approximately 10-m intervals across the transect.

   d. After taking the water quality measurements, grasp the end of the rope on the ponar sampler and lower the sampler to the bottom of the lake.

   e. Release the trip mechanism by sharply yanking up on the rope, and then pull the sampler to the surface of the water.

   f. While holding the sampler over the water and above the net of a Surber stream bottom sampler, open the jaws of the ponar sampler and allow the contents to fall into the Surber net. To insure that all material in the Ponar sampler is washed into the Surber net, repeatedly dip and raise the sampler in the water in the mouth of the Surber net until all visible signs of sediment and debris are gone from its inner surface.

   g. Wash the fine sediment and debris from the sample by repeatedly raising and lowering the Surber net in the water being careful not to submerge the frame of the net. Following the procedures for transferring a sample either directly from a Surber net (SOP-5, step 2j) or by tray (SOP-5, step 2k), transfer the sample to a sample jar containing both inside and outside labels with the site, date, sample number, and chain-of-custody number (see SOP-7, step 1a for deriving chain-of-custody numbers).

   h. Place an appropriately marked NFPA self-adhesive identification label on each jar; appropriate markings include chemical name (ethanol) and the health, flammability and reactivity numbers (1, 4, and 0, respectively, for ethanol). Place a self-adhesive "Radioactive Material" label on the exterior side of the jar.

   i. After all samples have been collected, place each sample jar into a shipping container; transport following the procedures in SOP-8.

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

j. Within one week after collection, replace the alcohol of each sample jar, and dispose of any liquid and solid low-level compactible wastes following the procedures in SOP-9.

k. At the end of each day, enter a summary of the day's sampling activities in a registered logbook maintained in the PI's office (Building 1505, Room 308). Additional information should also be included in the summary such as weather, unusual conditions, any problems encountered, etc.

Approval

PRINCIPAL INVESTIGATOR John B Smith DATE 2/25/92

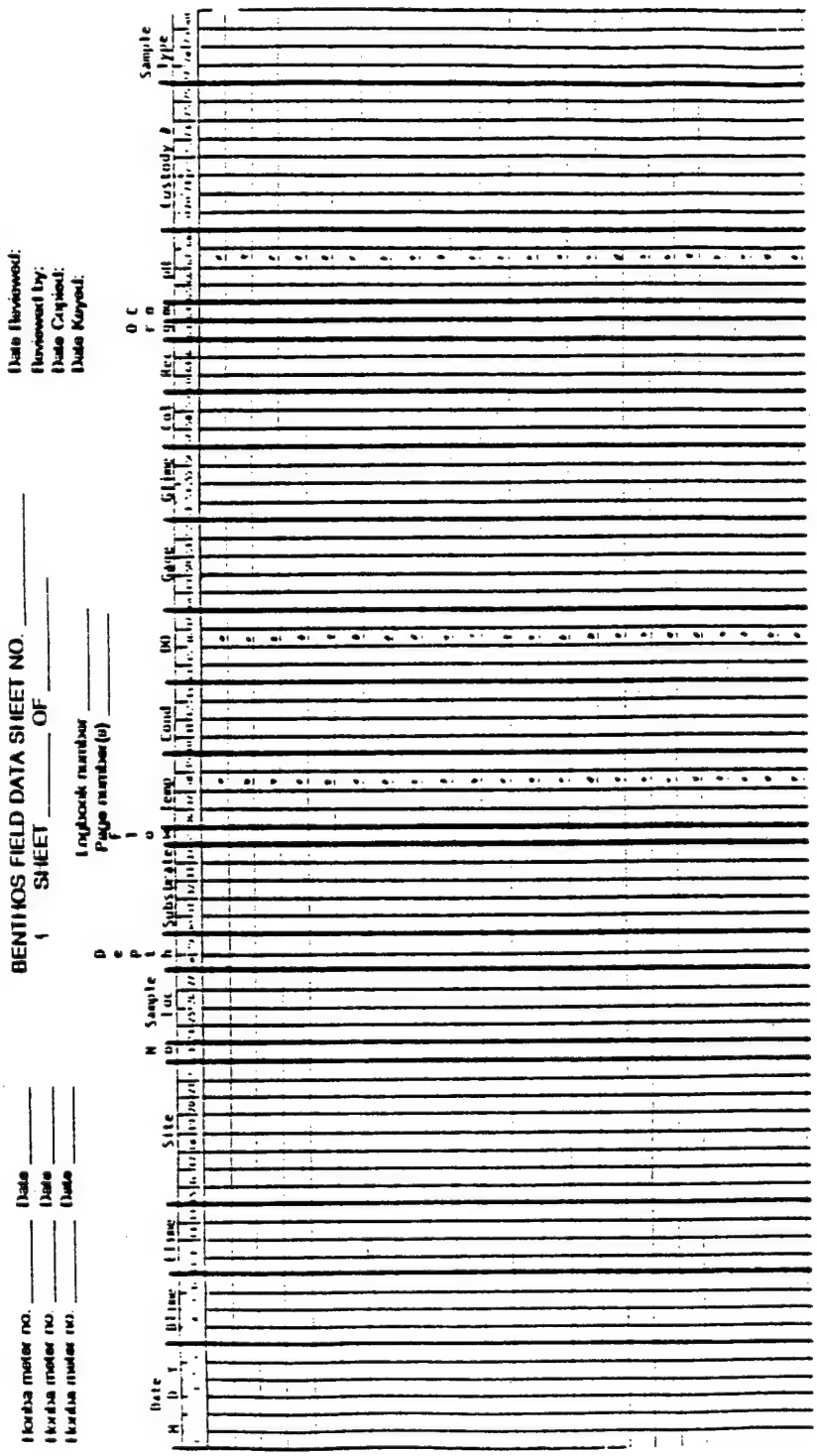
BENTHIC MACROINVERTEBRATE MONITORING
SAMPLE COLLECTION AND STORAGE

SECTION SOP-5
PAGE 8 of 10
DATE 10-31-91

BMAP QUALITY ASSURANCE PLAN

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

EXHIBIT 5-1



SUBJECT: QUANTITATIVE SAMPLE COLLECTION

EXHIBIT 5-2

Benthic Macroinvertebrate Field Data Sheet Instructions

Data Sheet Specific Variables	Instructions
Horiba Meter No.	Enter the I&C maintenance number from the Horiba meter along with the date used.
Benthos Field Data Sheet No.	Identification number for each data sheet comprised of a project identifier (BC = Bear Creek; EF = East Fork Poplar Creek; MI = Mitchell Branch; MC = McCoy Branch; PD = Paducah; PT = Portsmouth; WC = White Oak Creek watershed); date (four digits comprised of sampling month and year samples were collected); and data sheet page number separated from the rest of the ID number by a dash. Thus, the field data sheet number for data entered on page 2 for samples collected in White Oak Creek watershed on April 4, 1991 would be WC0491-2.
Logbook Number	Registration number for the Research and Technical notebook in which a daily summary of sampling activities for a specific project and sampling period are entered.
Page Number(s)	Page number(s) in Research and Technical notebook where the daily summary is written.
Date Reviewed	Date the field data sheet was reviewed for accuracy.
Reviewed By	Initials of person reviewing the data sheet.
Date Copied	Date a photocopy was made of the data sheet.
Date Keyed	Date the data were entered onto a computer.
Date	Month, Day, and Year (e.g., 080286 for August 2, 1986).
BTime	Time at which you begin sampling the station. Use 24 hr. clock (e.g., 1500 for 3:00 PM)
ETime	Time at which you stopped sampling the station. Use 24 hr. clock. If site is dry leave blank.
Site	Enter applicable site name as in SOP-1, Exhibit 1-1. Left justify names, and where enough spaces exist, leave a space between the "K" and the numerical portion of the name.

SUBJECT: QUANTITATIVE SAMPLE COLLECTION

EXHIBIT 5-2 (continued)

Data Sheet Specific Variables	Instructions
Sampno	Enter sample replicate number.
Samploc	Enter the transect and cell number for each replicate sample obtained following the procedures in SOP-4.
Depth	Depth (in centimeters) at which the sample is taken.
Substrate	Enter replicate sample substrate code obtained following step 1 in SOP-2.
Flow	Visual estimate of the flow rate. 1 = very slow; 2 = slow; 3 = moderate; and 4 = fast.
Temp	Water temperature in °C (right justify).
Cond	Conductivity in µs/cm (right justify).
DO	Dissolved oxygen in mg/L (right justify).
Gage	Staff gage reading taken once on each sampling date. East Fork Poplar Creek - Staff gage at bridge on Gum Hollow Road. Bear Creek - Staff gage at USGS Gaging Station at beginning of Bear Creek Road. White Oak Creek - Staff gage at lower most weir near mouth of Melton Branch. Melton Branch - Staff gage at weir near its mouth.
GTime	Time at which gage was read. Use 24 hr. clock.
Col	Initials of person taking the sample.
Rec	Initials of person recording data on field data sheet.
Org	Enter "1" when you observe organisms in the sample; leave blank if you see none present.
Com	Enter "1" when you have comment; leave blank when you have no comment. Enter comment on back of data sheet with site and sample number identified.
pH	Enter pH value.
Custody #	Enter chain-of-custody number from jar. (See SOP-7, step 1a).
Sample Type	Enter sample type where "N" = Quantitative and "L" = Qualitative (Left justify)

BENTHIC MACROINVERTEBRATE MONITORING SAMPLE COLLECTION AND STORAGE

SECTION SOP-6
PAGE 1 of 5
DATE 10-31-91

BMAP QUALITY ASSURANCE PLAN

SUBJECT: QUALITATIVE SAMPLE COLLECTION

Purpose

Provide procedures for the collection of qualitative macroinvertebrate samples from streams and reservoirs/ponds.

Equipment

95% denatured ethyl alcohol
16 oz polyurethane coated glass jars
Container for vehicle transportation of samples
1" X 1 1/2" self-adhesive labels
3/4" X 1" inner sample labels
"Radiative Material" labels
NFPA chemical identification labels
8" X 10" plastic white photo tray
Forceps
Shoulder length black neoprene-coated gloves
Horiba Model U-7 Water Quality Checker
D-Frame aquatic dip net (mesh size = 500 μm)
Two-gallon plastic bucket
Surber sampler with 368-μm mesh net
Hip boots or chest waders
Sample field data sheets (Exhibits 5-1 and 5-2)
Disposable latex laboratory gloves
Yellow C-area coveralls
Large yellow plastic bags (four)
Benthic macroinvertebrate sample chain-of-custody forms (Exhibit 7-1)
Clipboard or aluminum carrying case for field data sheets
No. 2 lead pencil
Pen with waterproof ink
Box for storing samples during transport (see SOP-8)
Portable radiation survey meter (beta/gamma)

Procedures

If samples are collected from a "Regulated Area", personnel shall wear appropriate protective clothing (see QA-BMAP-19-200). Each person shall wear C-area coveralls; the person collecting the samples shall wear shoulder length black neoprene gloves and the person processing the samples shall wear disposable latex laboratory gloves. Before reentering a vehicle and proceeding elsewhere all personnel shall scan themselves and their equipment, and handle any contaminated items per procedures in QA-BMAP-19-200.

BENTHIC MACROINVERTEBRATE MONITORING SAMPLE COLLECTION AND STORAGE

SECTION SOP-6
PAGE 2 of 5
DATE 10-31-91

BMAP QUALITY ASSURANCE PLAN

SUBJECT: QUALITATIVE SAMPLE COLLECTION

1. **Sample Collection - Stream Sites**

   a. Enter date, beginning time, station identification name, type of sample being collected (L = qualitative sample), and the initials of the sample collector and data recorder on the field data sheet (Exhibits 5-1 and 5-2).

   b. Prior to sample collection, obtain water quality measurements with an Horiba meter (see SOP-3). Enter data in appropriate spaces on the field data sheet.

   c. At each site, collect samples from a reach of approximately 50 m above and 50 m below the riffle from which routine quantitative samples are collected, being careful to avoid inclusion of this riffle if possible. Sample all visible habitats such as riffles, pools, leaf packs, undercut banks, runs (habitat with flows intermediate between those of riffles and pools), mosses, root hairs, submerged logs, emergent and submergent vegetation, etc. The amount of time spent taking a qualitative sample at each site with the aquatic kick net should be approximately 15 to 20 min.

   d. In those habitats having flowing water, face the mouth of the net upstream. While standing upstream of the net, disturb the stream bottom/substrate by moving your feet from side to side and with circular motions while moving backwards upstream, allowing the suspended material to drift into the capture net of the sampler.

   e. In those areas having little or no flow (e.g., pools, backwash areas near banks), disturb the substrate by foot and then pull the net through the suspended material two or three times to collect the suspended organisms.

   f. Sample root hairs along the edge of a stream by placing the sample net underneath a clump of roots and vigorously shaking the roots by hand (wearing shoulder length neoprene gloves) or by foot.

   g. Sample undercut banks by placing the net underneath the bank and rapidly pulling the net in and out from under the bank making sure that the suspended material passes into the net; the net should also be scraped against the top of the undercut bank to help dislodge clinging organisms.

   h. In addition to the use of the aquatic dip net to collect organisms, pick up several small to large rocks and submerged sticks/logs and examine them for clinging organisms (e.g., rock case building organisms), keeping several specimens of each type of organism seen; place any collected organisms into a sample jar containing 95% ethanol. Because some organisms burrow into wood as its ages in the water, they will move to the surface of the wood as it air drys; thus, the collected sticks should be briefly retained (approximately 10 to 15 min) and periodically examined while processing the sample collected in the dip net; place any collected organisms into the sample jar.

SUBJECT: QUALITATIVE SAMPLE COLLECTION

i. After collecting a sample with the aquatic dip net, fill a 2-gal bucket approximately one-third full of sample and then add about an equal volume of stream water to the bucket.

j. Swirl the sample in the bucket of water vigorously enough to suspend the lighter debris and lighter organisms.

k. While the material is still suspended, carefully pour the suspension through the Surber net being careful not to allow large pieces of debris to enter the net.

l. Repeat steps j and k above several times until the amount of material entering into suspension is minimal or the water no longer becomes turbid from the suspended material (approximately five to ten times).

m. Carefully remove and scan the coarse woody material (e.g., leaves, sticks, etc.) remaining in the bucket for organisms such as snails, mussels, and other organisms which build cases with sticks, leaves, and other heavy materials.

n. After scanning these woody materials, discard them back into the stream while leaving the heavier materials (i.e., rocks and gravel) in the bottom of the bucket. The material in the bottom of the bucket should be poured into a photo tray and scanned carefully for heavier organisms (e.g., snails, rock case building organisms) which may not have been dislodged with the above methods. After scanning this material, discard it into the stream.

o. Repeat steps i through n until all material in the kick net has been processed.

p. Transfer the material and organisms washed into the Surber net into a polyurethane coated glass sample jar by following the steps 10 or 11 of SOP-5. If the sample requires more than one jar, the appropriate information should be reflected on the labels (i.e., jar 1 of 2 and jar 2 of 2, etc., and the information as indicated in step r below), and noted on the field data sheet in the comment section.

q. If necessary, add additional alcohol to the sample jar, ensuring that all material is covered with liquid.

r. Place labels having the site name, collection date, and chain-of-custody number (see SOP-7, step 1b for deriving chain-of-custody numbers) both inside and outside of the sample jar. Additionally, place an appropriately marked NFPA self-adhesive identification label on each jar; appropriate markings include chemical name (ethanol) and the health, flammability, and reactivity numbers (1, 4, and 0, respectively, for ethanol). If the sample has been collected from an area potentially contaminated with radionuclides, place a self-adhesive "Radioactive Material" label on the exterior side of the jar.

s. After each sample has been collected, package it in a sample container box for transportation (see SOP-8). Replace the alcohol of each sample replicate within one week after collection and dispose of any liquid and solid low-level compactible wastes following the procedures in SOP-9.

2. **Sample Collection - Reservoirs/Ponds**

a. Enter date, beginning time, station identification name, type of sample being collected (L = qualitative sample) and the initials of the sample collector and data recorder on the field data sheet.

b. Before collecting a sample, obtain a water quality measurement with the Horiba meter (see SOP-3). Enter data in appropriate spaces on the field data sheet.

c. Qualitative samples taken from reservoirs and ponds will key on the collection of those organisms living in the shallow water areas (i.e., littoral zone). Sample all visible habitats such as rock riprap, submergent and emergent vegetation, old trees and logs that have fallen in the water, and other dead vegetation, such as leaf packs along the shallow banks. etc.

d. In soft substrates, scrape the net across the surface while applying a slight amount of pressure so that the upper few centimeters of sediment will be forced through the net.

e. In areas with much organic debris (e.g., leaves and leaf fragments), repeatedly raise and lower the net to suspend the debris and associated organisms. Then rapidly pull the net through the suspended material two or three times to collect the suspended organisms.

f. For emergent and submergent vegetation, sweep the net rapidly through the vegetation several times to dislodge, suspend, and collect the attached organisms.

g. Collect and gently rub several large rocks from rock riprap to dislodge the attached organisms into the collection net. Discard rocks back into the water.

h. Sample areas containing small gravels/rocks in a manner similar to areas containing a soft substrate (step d).

i. If small logs/large sticks are available, remove and visually scan them for organisms. After the first examination, allow the logs/large sticks to air dry and periodically examine them over a 10 to 20 minute period for additional organisms. Collect representative specimens of each type of organism.

SUBJECT: QUALITATIVE SAMPLE COLLECTION

j. Sample dead emergent type vegetation (i.e., trees and shrubs) by rubbing the sample net frame up and down along the branches and main trunk to dislodge attached organisms. Sweep the net back and forth through the dislodged material to collect suspended organisms.

k. After collecting the sample, process and transfer the material and organisms, and package the sample following steps 1i through 1s of SOP-6.

Approval

PRINCIPAL INVESTIGATOR J. B. Smith DATE 2/25/92

Appendix B: Standard Operating Procedure for *Arabidopsis thaliana* 21-Day Seed Germination and Shoot Biomass Soil Toxicity Test

DRAFT STANDARD OPERATING PROCEDURE

revised 3/15/94

SUBJECT: *Arabidopsis thaliana* 21-d seed germination and shoot biomass soil toxicity test.

Purpose

To measure the toxicity to *Arabidopsis thaliana* of test soils by germination and shoot biomass production

Equipment

Promix® potting soil
Test soils, including a reference soil
Arabidopsis thaliana (var. Columbia wild-type) seeds
plastic disposable sterile Petri dishes (100mm X 20mm) with covers
Distilled water
Environmental chamber capable of maintaining 25±2° C and 350±50 μmol/m^2/sec PAR
Forceps
Labels
Spray bottle
Laboratory gloves
soft bristled, small paint brush
Scissors
Coin envelopes
Resealable polyethylene bags--1 pint capacity (e.g. Ziplock®)
Needle or similar puncturing device
Balance accurate to 0.01 mg
Balance accurate to 10 mg
Drying oven capable of maintaining 65±5° C
Drying oven capable of maintaining 100±5° C
White plastic photographic trays (12" X 18" X 2")
Weighing tray
***Arabidopsis* Test Logbook** (registered)
***Arabidopsis* Test Data Logsheets** (exhibit 5)
Soil Toxicity Test–Soil Samples Datasheets (exhibit 1)
Soil Toxicity Test–Moisture Fraction Worksheets (exhibit 2)
Soil Toxicity Test–Equipment Datasheet (exhibit 3)
***Arabidopsis* Test–Watering Logsheet** (exhibit 4)
Heavy white paper
Scintillation vials
Spatula

Procedure

1. Before handling any suspect or hazardous soils, determine appropriate safety precautions. These precautions always include the use of chemically impermeable, laboratory gloves and laboratory safety glasses. Additional measures may include the use of respirators, fume hoods, or radiation protection equipment. The Principle Investigator (P.I.) of the study will determine appropriate safety measures for the particular soils to be tested. The P.I. will also determine the appropriate waste disposal measures needed for used test soils in accordance with ORNL waste disposal guidelines.

2. Record all test soil identification information on the **Soil Toxicity Tests–Soil Samples Data Sheet** for all soil samples to be tested. Record identification information for environmental chambers, balances, ovens and any other electronic equipment on **Soil Toxicity Tests–Equipment Data Sheets**, including whether the equipment must be user-calibrated.

3. From among all soils (including reference and Promix®) choose one soil to set up and estimate the amount of soil needed to set up five petri dish replicates (about 500 cm^3). Pour this amount of soil into a photographic tray. Soils should be set up in random order. Randomization may be accomplished in several ways (e.g. by generating random numbers which correspond to soil container numbers) and should be documented in the ***Arabidopsis*** **log book**.

4. Elevate moisture content of the test soil to a suitable level for seed germination. This procedure is necessarily subjective, and depends upon the judgement of a trained laboratory technician. Using defined hydrations such as 75% of water holding capacity (WHC) for all soils (Green et al. 1989) has proven unsatisfactory in our experience due to variability in soil make-up under these conditions. Suitable moisture level is attained by adding distilled water to the soil sample, and mixing the soil thoroughly by hand, until the soil texture is that of a thick mud. Any solids found in the soil during mixing that are greater than 1 cm. in diameter should be removed and returned to the soil sample container. Promix® has proven to be suitable for hydrating to 75% water holding capacity, based on a WHC of 5 ml/g of dry soil.

5. Fill three pre-weighed scintillation vials with the hydrated soil. Label the vials with the soil container and replicate number, determine the mass of wet soil in each vial, and record this data on the **Soil toxicity test–moisture fraction worksheet**. Once vials for all test soils to be set up in a day have been obtained, place the vials into a drying oven at 100±5°C. Allow the soil to dry for >48 h, remove the vials, allow them to cool to room temperature in a dessicator, then weigh each vial on a balance accurate to 10 mg. Record this weight on the **Soil toxicity test–moisture fraction worksheet**.

Soil Moisture Fraction=(Hydrated Soil Mass-Dried Soil Mass)/Hydrated Soil Mass

6. Divide the hydrated soil among five Petri dishes until a soil depth of approximately 1 cm. is attained. A spatula may be used to transfer soil and to ensure even coverage of the dish surface, however the soil should not be compressed or packed. Label each dish with the container number and replicate number (1-5). Return unused soil to its original container. Spray the soil in each dish once with a spray bottle containing distilled water.

7. Gently tap the container of *Arabidopsis thaliana* seeds over a sheet of clean white paper with a single crease. Using a soft paintbrush, separate 20 seeds and return excess seeds to the seed

container. Hold the paper over a dish and distribute the seeds evenly over the dish, either be tapping the paper or using the paintbrush. Tape a cover securely on the dish. Repeat this step for each replicate.

8. Repeat steps 3-7 until all soils to be set up have been inoculated with seeds.

9. Place all Petri dishes in the environmental chamber so that the petri dish lies flat. The environmental chamber should be set at 25±2°C on a 14-h/10-h day/night photoperiod with a light intensity of 350±50 μmol/m^2/sec PAR.

10. On day T_7 (T_0 is the day that seeds are added to soil) remove the petri dishes from the environmental chamber. One at a time, remove the lid from a dish. Count and record the number of germinating plants in each petri dish on the ***Arabidopsis*** **Test Data Logsheet**. Germination is defined as the appearance of the plant coleoptile above the surface of the soil medium. Germination must be >90% in the Promix® (the negative control) for the test to be considered valid (Greene et al. 1989).

11. Thin plants to a density of 10 per dish by plucking out excess plants with forceps. Remove plants that are clumped together first, leaving the larger plant, then remove the smallest of the remaining plants, and finally, remove plants haphazardly until ten plants remain. Place the open dish in a resealable polythylene bag which has been labeled with the soil container and replicate number and punctured with a clean needle or similar tool twenty times over the bag's surface area. Arrange the dish so that it can sit flat with the bag standing upright. Fold the bottom corners of the bag underneath the dish and tape them in place. Spray each dish in its bag five times with distilled water from a spray bottle. Seal each bag and replace it in the environmental chamber.

12. From day T_8 to day T_{20}, the dishes should be monitored every day. If conditions within a bag are dry (e.g. dry soil surface, or no condensation on inside of bag), open the bag and spray distilled water over the Petri dish five times (or more if the soil appears very dry). Document monitoring information on the ***Arabidopsis*** **Test Watering Logsheet**.

13. On day T_{21}, remove containers from the environmental chamber. One at a time, harvest each replicate by removing the Petri dish from its bag and, using forceps, separate the plant tissue from the soil by gently pulling on the plant. Once the plant is freed from the soil, cut away any attached below ground tissue with a scalpel (Unpigmented tissue below the basal rosette leaves is below ground tissue). Remove any soil particles adhering to the plant with a soft paintbrush. Place all above-ground tissue from a replicate dish into a coin envelope and label the envelope with the soil container and replicate number. Record the number of plants harvested in each replicate ***Arabidopsis*** **Test Data Logsheet**. Repeat this procedure for all T_{21} replicates. Place all envelopes into a drying oven at 65±5°C.

14. Allow the plant material to dry for >48 h. Remove the envelopes from the oven and place into a dessicator to cool for at least one hour. Determine the mass of each envelope's contents using a balance accurate to 0.01 mg. Record the dry biomass on the ***Arabidopsis*** **Test Data Logsheet**.

15. Data from the *Arabidopsis* test may be entered on a computer spreadsheet, from which the the fraction of seeds germinating on T_7 per replicate, the mean above-ground biomass per plant within each replicate and the mean soil moisture fraction of each soil sample may be calculated.

Green, J. C., C. L. Bartels, W. J. Warren-Hicks, B. R. Parkhurst, G. L. Linder, S. A. Peterson, W. E. Miller. 1989. Protocols for Short Term Toxicity Screening of Hazardous Waste Sites. United States Environmental Protection Agency. Environmental Research Laboratory. Corvallis. OR 97333. EPA/600/3-88/029.

Soil Toxicity Test—Soil Samples Datasheet

Soil for use in : (test name)________________________ Test No.: __________

Data recorded by: ______________

Site ID	Sample Identification Number	Moisture fraction	Container #

Soil Toxicity Test–Moisture Fraction Worksheet

Test ID: ______________________ time/date into oven: ________ oven id: ________

data recorded by: ______________ time/date out of oven: ________ oven temp: ____

sample id #	vial #	vial mass (g.)	hydrated soil mass (g.)	dried soil mass (g.)	moisture fraction	mean moisture fraction

Balance calibration:

date of use	equipment id	mass of 10.0000 g. reference	initials
________	________	________________	______

Soil Toxicity Tests–Equipment Datasheet

Equipment used for: (test name)_____ Test start date: _____ Page ___ of ___

Data recorded by: _____

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Arabidopsis Test–Watering Logsheet

Test name: ________________ Test dates: from _____ to _____

Environmental Chamber serial number(s): ____________________

Date of watering	Initials

Arabidopsis Test Data Logsheet

Test ID: _____ Test initiation date: _____

Data recorded by: _____ Environmental Chamber serial #: _____

container #	rep	T_0 germ	T_{21}: # plants	total dry mass (g.)

Balance calibration:

date of use	equipment id	mass of 1.0000 g. reference	initials
_____	_____	_____	_____

Appendix C: Standard Operating Procedure for *Eisenia foetida* Chronic Soil Toxicity Test

DRAFT STANDARD OPERATING PROCEDURE

revised 3/15/94

SUBJECT: *Eisenia foetida* chronic soil toxicity test

Purpose

To evaluate the effects of soil contaminants on the growth, mortality and reproductive vigor of the earthworm, *Eisenia foetida*.

Equipment:

Eisenia foetida--clitellate adults
Glass finger bowls > 250 ml. capacity
Environmental chamber capable of maintaining 20 ± 2°C
Distilled water
Thermometer
Bent nose forceps
Resealable polyethylene bags (e.g. Ziplock® sandwich bags, 6.5"X5.9")
Artificial soil with finely ground peat moss (see step 3 for ingredients)
Fermented alfalfa food substrate (see step 2 for ingredients)
Phototray
Balance (accurate to 10 mg)
Brass sieve--30 cm. diameter, mesh size between 840 and 500 µ
Paper towels
Spatula
Scintillation vials
Drying oven capable of maintaining 100 ± 5°C
80 ml. beakers
Grinding mill
High pressure shower nozzle, or similar water spraying device
Coin envelopes
Earthworm Test Initiation–Data Logsheet (exhibit 1)
Earthworm Test–T_{21} Data Logsheet (exhibit 2)
Soil Toxicity Test–Moisture Fraction Worksheet (exhibit 3)
Soil Toxicity Test–Equipment Datasheet (exhibit 4)
Soil Toxicity Test–Soil Samples Datasheet (exhibit 5)
Earthworm Tests Logbook (registered)

Procedure

1. Chemically resistant laboratory gloves should always be worn when handling *Eisenia fetida*. Additional safety precautions, such as the use of safety glasses, respirators, and fume hoods may be necessary when handling soils or potentially hazardous waste. The principal investigator (P.I.) of the test will determine the appropriate safety measures needed. Likewise, the P.I. will determine the appropriate means of waste disposal for soil samples in accordance with ORNL guidelines.

2. At least two weeks before initiation of test, fermented alfalfa food substrate must be prepared. Existing fermented alfalfa used in cultures may be used for the test, provided the dry mass and water content of the alfalfa is known. To make fermented alfalfa, add a known mass of dried alfalfa pellets to a one liter container so that approximately ½ of the container's volume is occupied. Fill the container with distilled water, measuring the amount of water added. Seal and label the container, including dry mass of pellets, water volume added, date prepared, and preparer's name on the label. Allow the alfalfa to ferment for at least 14 days before using it in a test.

3. At least 3 kg artificial soil will be needed for use as a negative control soil (for 30 replicates). To prepare artificial soil, thoroughly mix the following dry ingredients on a percent dry weight basis.

-- dry silica sand, sieved through a 500μ mesh screen--70%
-- kaolinite clay, sieved through a 500μ mesh screen--20%
-- sphagnum peat moss, ground in a mill to pass a 2mm screen--10%

Determine the pH of the artificial soil, using method 9045A (see exhibit 4 for procedure and equipment). If pH is below 6.0, calcium carbonate should be mixed with the soil to a concentration no greater than 3% of total dry weight. The acceptable pH range for artificial soil is 6.0-8.0 (Green et al. 1989). Label the container of artificial soil with the date prepared, preparer's name, ingredients with % composition and pH.

4. The day prior to test initiation, harvest clitellate adult *Eisenia foetida* specimens from cultures following harvesting procedures in step 9 of SOP-1. 20 earthworms will be required for each soil sample, including reference soil(s), plus 60 earthworms for the artificial soil control. Harvested worms should first be rinsed in a bowl of distilled water, then placed in groups of 10-12 into finger bowls containing approximately 250 ml of 20 ± 2°C distilled water. Each bowl should be covered with a paper towel, then placed into an environmental chamber set at 20 ± 2°C. Earthworms should be removed from the distilled water within 12-24 h. Earthworms left in water longer than 24 h should be carefully inspected for signs of stress (swollen areas, lack of response to touch) before use.

5. Record test soil identification information on the **Soil Toxicity Test–Soil Samples Datasheet** for all soil samples to be tested.

6. Record identification information for environmental chambers, balances, ovens and any other electronic equipment to be used on the **Soil Toxicity Test–Equipment Datasheet**. Include how the instrument is calibrated.

7. From among all soils (including reference and artificial soils)) choose one soil to set up and pour aproximately 1.5 kg of this soil into a photographic tray. If the Arabidopsis soil toxicity test is to be set up simultaneously with this test, additional soil should be poured into the photographic tray (see SOP 3). Soils should be set up in random order. Randomization can be accomplished in several ways (e.g. by generating random numbers which correspond to soil container numbers) and the method should be documented in the **Earthworm Test Logbook**.

8. Elevate moisture content of this test soil to a suitable level for earthworm survival. This procedure is necessarily subjective, and depends upon the judgement of a trained laboratory

technician. Using defined hydrations such as 75% of water holding capacity (WHC) for all soils (Green et al. 1989) has proven unsatisfactory in our experience due to variability in soil make-up under these conditions. *Eisenia foetida* tolerate a wide range of moisture levels and soil toxicity appears to be unaffected by small variations (Heimbach and Edwards. 1983). Suitable moisture level is attained by adding distilled water to the soil sample and mixing the soil thoroughly by hand. until the soil texture is that of a thick mud. Any solids found in the soil during mixing that are greater than 1 cm in diameter should be removed and returned to the soil sample container.

9. Fill three pre-weighed scintillation vials with the hydrated soil. Label the vials with the sample identification number. determine the mass of wet soil in each vial. and record this data on the **Soil toxicity test–moisture fraction worksheet.** If this test is being set up simultaneously with the *Arabidopsis* soil toxicity test. this step is identical to step 4 of SOP-3. and need not be repeated. Once vials for all test soils to be set up have been obtained. place the vials into a drying oven at 100 ± 5°C. Allow the soil to dry for >48 h. remove the vials. allow them to cool to room temperature in a dessicator. then weigh each vial on a balance accurate to 10 mg. The balance must be calibrated before use. and this action documented on the **Soil toxicity test moisture fraction worksheet.** Record the data and determine and record each soil's moisture fraction on the **Soil toxicity test–moisture fraction worksheet.**

Moisture fraction=(Hydrated Soil Mass-Dried Soil Mass)/Hydrated Soil Mass

10. Using a needle. or similar puncturing device. puncture ten resealable plastic bags twenty times evenly over each bag's surface. Label each bag with the soil container and replicate number (1-10).

11. Using a spatula. or a glove-protected hand. fill each bag with 150 ± 5 g. of hydrated soil.

12. Place a 1.0 g clump of fermented alfalfa onto the soil surface in each bag.

13. Remove two fingerbowls containing earthworms and distilled water from the environmental chamber. Weigh the hydrated earthworms two at a time. The worms should be scooped from the water with bent nose forceps. placed onto a piece of filter paper to draw off excess surface moisture for a few seconds. then placed on the balance in a tared weighing tray Because the worms rapidly lose moisture when exposed. the first stable mass should be recorded on the **Earthworm Test Initiation–Data Logsheet.** otherwise the mass reading will steadily decrease as the earthworms' moisture evaporates. Gently remove the worms from the balance and place the couple onto the soil surface of their corresponding bag. Repeat this step until all replicate bags have been filled. If any earthworm in a fingerbowl is obviously stressed. or dead (swollen or ruptured clitteli are typical indications), do not use any of the earthworms from that fingerbowl.

14. Repeat steps 7-13 until every test soil has been set up.

15. Place all bags into an environmental chamber. allowing a slight spacing between bags for air exchange. The chamber temperature should be constant at 20 ± 2°C with a 12 hour day lighting schedule. Light intensity may vary

16. Twenty-one days after the test is initialized. remove the test bags from the environmental chamber.

17. Empty the contents of one bag into a phototray, rinse the emptied bag to remove adhering soil and pour the rinsate into the phototray. Search the soil for surviving adult earthworms, adding more water to disolve clumped soil if necessary. Rinse survivoring worm pairs in distilled water, then place them into an 80 ml beaker with approximately 60 ml of 22 ± 5°C distilled water. If only one worm has survived, it may be discarded since its individual mass change cannot be determined. Label the beaker with the soil container and bag replicate number and cover the beaker with a paper towel. Record the number of recovered adult earthworms on the **Earthworm Test–T_{21} Data Logsheet**. Mortality in the artificial soil control must be < 10% for the test to be considered valid (Greene et al. 1989).

18. Pour the soil and water from the phototray into a brass sieve (between 840 and 500µ mesh). With a high pressure shower nozzle, rinse the soil through the sieve. Invert the sieve over a phototray and rinse the remaining matter from the sieve into the phototray. Carefully inspect the debris for unhatched cocoons. Remove the cocoons, place them into a coin envelope, label the envelope with soil container and replicate number, and record cocoon number on the **Earthworm Test–T_{21} Data Logsheet**.

19. Repeat steps 17-18 for all T_{21} bags. Place the beakers containing surviving adult pairs into an environmental chamber at 20 ± 2°C.

20. Remove the beakers within 12-24 h. Transfer the worms from each beaker into a numbered, pre-weighed scintillation vial. Label the vial with the soil container and replicate number (vial label and mass should be recorded on the **Earthworm Test–T_{21} Data Logsheet**). Cover each vial with aluminum foil to prevent earthworm escape. Place the vials into an oven at 100 ± 5°C and allow the worms to dry for > 48 h (Record time into and out of the oven on the **Earthworm Test–T_{21} Data Logsheet**). Remove the vials and allow them to cool in a dessicator. Determine the final dry mass of the worm pairs using a calibrated balance accurate to 10 mg and record on the **Earthworm Test–T_{21} Data Logsheet**.

21. Raw data may be entered onto a computer spreadsheet (e.g. Lotus 1.2.3) on a personal computer, from which calculations can be made (e.g. survival fractions, soil moisture fractions). The change in adult dry mass per couple over the course of the test may be determined by estimating the initial dry mass of the adult pair. This estimation is based on a regression of wet weight on dry weight for 49 individuals subjected to conditions described in step 4, and ranging in initial fresh weight from 113 mg to 623 mg (R^2=0.9851, SE=0.001736, intercept forced through zero).

$$\text{DRY MASS CHANGE} = 0.097871 * T_0 \text{ WET MASS} - T_{21} \text{ DRY MASS}$$

Methods of statistical analysis may vary with sampling design and data obtained. Appropriate methods will be determined and documented by the P.I. using available literature.

Literature cited

Green, J. C., C. L. Bartels, W. J. Warren-Hicks, B. R. Parkhurst, G. L. Linder, S. A. Peterson, and W. E. Miller. 1989. Protocols for Short-Term Toxicity Screening of Hazardous Waste Sites. United States Environmental Protection Agency, Environmental Research Laboratory, Corvallis, OR. EPA/600/3-88/029.

Heimbach, F. and P. J. Edwards. 1983. The Toxicity of 2-Chloroacetamide and Benomyl to Earthworms Under Various Test Conditions in Artificial Soil Test. Pesticide Science. 14:635-636.

Earthworm Test Initiation—Data Logsheet

test name: _____ test date: _____

Data recorded by: _____

Soil Sample ID #	Container #	Replicate #	Test Day #	Initial fresh mass of earthworm pair

Balance ID:_____ Mass of 10.0000 g. reference mass:_____

Earthworm Test—T_{21} Data Logsheet

Test ID: ________ Data recorded by: ________ T_{21} date: ________

soil id	vial id (container#-rep)	vial mass (g)	# live adults	# cocoons	vial+dried adults (g)	cocoon dry mass (g)

Balance ID	Date	Mass of 10.0000 g. reference	initials

Oven ID	Date/time in	Date/time out	temperature	initials

Soil Toxicity Test—Moisture Fraction Worksheet

Test ID: ____________________ time/date into oven: __________ oven id: __________

data recorded by: ____________________ time/date out of oven: __________ oven temp: _____

sample id #	vial #	vial mass (g.)	hydrated soil mass (g.)	dried soil mass (g.)	moisture fraction	mean moisture fraction

Balance calibration:

date of use	equipment id	mass of 10.0000 g. reference	initials

Soil Toxicity Tests—Equipment Datasheet

Equipment used for: (test name)_____ Test start date: _____ Page ___ of ___

Data recorded by: _____

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Instrument description: _____

Model: _____ Serial #: _____

Location: _____ Local ID # (if any): _____

Calibration information (i.e. frequency, responsible party, etc.):

Soil Toxicity Test—Soil Samples Datasheet

Soil for use in : (test name)____________________ Test No.: ________

Data recorded by: ______________

Site ID	Sample Identification Number	Moisture fraction	Container #

Appendix D: Standard Operating Procedures for Toxicity Tests for Aquatic Biota

ESD TOXICOLOGY LABORATORY

QUALITY ASSURANCE MANUAL

SECTION SOP-9
PAGE 1 of 16
DATE 10-01-93

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Purpose

To measure the chronic toxicity of water samples to fathead minnows during 7-d static renewal exposures.

Reference

EPA Test Method 1000.0, in C. I. Weber et al., *Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms*, EPA/600/4-89/001 (March 1989).

Equipment

Fathead minnow larvae (see SOP-6)
Temperature-controlled water bath or environmental chamber (maintained at 25 ± 2°C)
Dilute mineral water (DMW; see SOP-1)
Microbalance accurate to 0.0001 mg (e.g., Cahn)
Microweight aluminum pans (e.g., Cahn)
Drying oven
10- and 600-mL beakers
250-mL glass beaker or a crystallizing dish
Volumetric flasks
Graduated cylinders
Fixed- or adjustable-volume pipettes
Disposable pipette tips
Various colored stickers (for color-coding beakers)
Polished glass tubing (2-mm ID)
1-mL pipette bulbs
Siphoning hose with modified Y-shaped tygon connector
White plastic photographic tray
1-oz disposable polystyrene beakers
8-oz specimen containers (or equivalent)
Newly hatched brine shrimp (see SOP-5)
Perforated aluminum foil
Desiccator
Scintillation vial
Formaldehyde solution
Disposable gloves
Fine mesh aquarium net
Tweezers (or equivalent)
Registered fathead minnow logbook
Registered test organism shipment logbook (if required)
Fathead Minnow Test Information Logsheet (exhibit SOP-9.1)
Fathead Minnow Daily Test Information Logsheet (exhibit SOP-9.2)
Fathead Minnow Daily Test Results Logsheet (exhibit SOP-9.3)

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Fathead Minnow Shipment Logsheet (exhibit SOP-9.4)
Fathead minnow Weight and Survival Data for Lotus Program Logsheet (exhibit SOP-9.5)
Lotus 1-2-3 computer program

Procedure

1. The following tasks shall be performed in preparation for the test.

   a. Prepare the glassware. Label and color code four 600-mL beakers per test concentration (or site). Each of these four will be replicates (REPs). Label the necessary graduated cylinders and volumetric flasks.

   b. Prepare the weight beakers for the test larvae. Label 10-mL beakers with the sample, dilution, and REP number. Predry microweight pans in a 100 $\pm$ 2°C oven for at least 2 h. Place a microweight pan in each 10-mL beaker, and put the beakers in a suitable container (e.g., a 250-mL glass beaker or a crystallizing dish, depending upon the number of 10-mL beakers needed). Weigh the microweight pan from each beaker on a calibrated microbalance, and record the initial weight of each pan on the "Fathead Minnow Weight and Survival Data for Lotus Program" logsheet. Use tweezers (or equivalent) to handle microweight pans. Put each microweight pan back in its appropriate beaker. Cover the container of beakers with perforated foil, and store in a sealed desiccator. Store the logsheet in a safe location until the test is over.

   c. Prepare the weight beakers for the initial larvae. Label four 10-mL beakers as "initial 1," "initial 2," "initial 3," and "initial 4." These beakers will be used to find the weight of the larvae that initiate the test. Place a microweight pan in each 10-mL beaker, and weigh as described in step 1b.

   d. Check the number of in-house larvae available for the toxicity test. Only if necessary, order larvae from an approved outside source. The Laboratory Steward or designee is responsible for ordering larvae needed for conducting toxicity tests.

      (1) If larvae are ordered from an outside source, the following steps are performed.

         (a) Glue a "Fathead Minnow Shipment Logsheet" (exhibit SOP-9.4) into the registered test organism shipment logbook. Initial and date the logsheet so that half of the writing extends onto the logbook page.

         (b) Record the larvae source, approximate number of larvae received, date larvae received, and your initials. Also record the initial temperature of the water the larvae were shipped in, the time the temperature was taken, the thermometer number, your initials, and any observations or comments (i.e., condition of larvae).

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

(c) Measure the temperature of the water hourly to record the acclimation of the test organisms. Record the necessary information on the logsheet until the end of the workday or until the water reaches ~25°C.

e. Prepare brine shrimp according to SOP-5.

2. To start the test do the following.

a. Glue a "Fathead Minnow Test Information" logsheet, a "Fathead Minnow Daily Test Information Logsheet," and a "Fathead Minnow Daily Test Results" logsheet into the registered fathead minnow logbook. Initial and date each logsheet so that half of the writing is on the logsheet and half extends onto the logbook page. Note: more than one daily test results logsheet may be needed for a test.

b. Isolate the larvae. Using a polished glass tube and pipette bulb, transfer ten larvae that are <24 h old from the hatching chamber to a 1-oz polystyrene beaker containing 5-8 mL of control water. Transfer ten larvae for every REP of the test. Also transfer at least ten larvae for each initial beaker.

c. Begin the test. Randomly transfer larvae from the 1-oz polystyrene beakers to 600-mL beakers containing about 250 mL of test solution. The amount of water transferred with the larvae should be kept to a minimum to avoid dilution of the test solution.

d. Randomly arrange test beakers in a temperature-controlled water bath or environmental chamber (25 ± 2°C).

e. Feed the larvae in each test beaker 100 µL (~1500 shrimp) of newly-hatched brine shrimp twice daily at a ~8 h interval (at the beginning and at the end of the work day). Record the feeding time on the "Fathead Minnow Daily Test Information Logsheet."

f. Record the test name, test number, test dates, sites/concentrations tested, control water type, source of test larvae, date larvae hatched, and your initials on the "Fathead Minnow Test Information" sheet.

g. Record the date, the time the first test beaker received its larvae, the time the last test beaker received its larvae, test name, test number, test dates, test beaker and water bath or chamber temperatures, thermometer number, control water batch number, and your initials on the "Fathead Minnow Daily Test Information Logsheet."

h. Record the test name, test number, test dates, and sites/concentrations tested on the "Fathead Minnow Daily Test Results" logsheet.

i. Transfer all the larvae from the initial beakers into a scintillation vial containing dechlorinated tap water. Add several drops of formaldehyde solution. The scintillation

vial will now contain at least 40 larvae. Label the vial with "initials," the date and the name of the test. Store the vial in a safe location until the end of the test.

3. Repeat the following renewal process every day, starting 24 h after the test begins and continuing until the test is finished on Day 7.

   a. Use fresh samples and make fresh dilutions.

   b. Feed the larvae 100 μL of newly-hatched brine shrimp a minimum of 2 h prior to solution renewal. Record the feeding time on the "Fathead Minnow Daily Test Information Logsheet."

   c. Measure the temperature in two test beakers and record on the "Fathead Minnow Daily Test Information Logsheet." Also record the water bath or chamber temperature and thermometer number.

   d. Prior to solution renewal, carefully pour test water (~180 mL total) from at least two REP beakers into a labelled beaker or plastic cup. This water will be used to determine the final pH and dissolved oxygen concentration. NOTE: test water will only need to be collected from sites or concentrations in which water chemistry is being performed.

   e. Change the water in all REP beakers for a particular concentration (or site) before starting the next four-beaker series.

      (1) Siphon off old water, excess shrimp, and detritus from the beakers, using rubber tubing and a modified foot made from a Y-shaped tygon connector. Slowly siphon the water from the beaker into a white plastic photographic tray until ~50 mL of old test solution remains. Control the flow through the siphon by holding one gloved finger over the end of the tubing.

      (2) If any larvae are accidentally siphoned off with the water, retrieve them from the plastic tray, using a polished glass tube and pipette bulb. Then return them to the beakers.

      (3) Record the number of larvae surviving in each beaker, the total number of larvae alive for each concentration, and any appropriate code (see the "Fathead Minnow Daily Test Results" logsheet for key to codes) on the "Fathead Minnow Daily Test Results" logsheet in the registered fathead minnow logbook. Discard the dead larvae.

      (4) Add ~250 mL of fresh sample to each beaker. Add the fresh solution very slowly, gently pouring it down the side of the beaker to avoid disturbing the larvae.

   f. Record minor test non-conformities (e.g., a temperature that falls outside the acceptable range), on the "Fathead Minnow Test Information" logsheet.

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

g. Feed the larvae 100 µL of newly-hatched brine shrimp ~8 h after the first feeding. Record the feeding time on the "Fathead Minnow Daily Test Information Logsheet."

4. Method for terminating the fathead minnow chronic toxicity test when performed in the Toxicology Laboratory, Room 12, Building 1504.

a. Terminate the test after the fish have been exposed to the test solution for 7 consecutive days ± 2 h.

b. Siphon out as much of the test solution as possible from a REP beaker and pour the remaining solution (containing the surviving larvae) through a fine mesh aquarium net. Carefully rinse the larva with distilled water to remove any excess food or detritus, if necessary.

c. Kill the larvae that are retained on the net by severing their spinal cords with forceps.

d. Obtain the corresponding 1-mL weight beaker prepared in step 1b. Remove the microweight pan from the beaker. Using forceps, remove the larvae from the net and place on the microweight pan. Return the pan to the beaker. Place the beaker in a suitable container. Continue this procedure for the remaining REP beakers.

e. Obtain the scintillation vial containing the initial larvae. Pour the contents of the vial through the aquarium net to obtain the larvae. You may need to rinse the vial several times with distilled water to remove all the larva. After all the larvae are on the net, rinse the larva several times with distilled water to remove the formaldehyde solution. Remove ten larvae from the net and place on the preweighed microweight pan from an initial beaker. Repeat this procedure for all four initial beakers. Discard the waste formaldehyde solution in an appropriately labeled bottle located in the fume hood in Room 11, Building 1504.

f. Cover the container holding the beakers with the microweight pans with perforated aluminum foil. Place the container in a drying oven and let the contents dry a minimum of two hours at 100 ± 2°C.

g. Remove the container from the oven and place in a desiccator a minimum of four hours to cool the larvae before weighing them on a calibrated microbalance.

h. Record the final pan weights and the number of larvae surviving in each beaker on the "Fathead Minnow Weight and Survival Data for Lotus Program" logsheet that was prepared in step 1.b.

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

5. Method for terminating the fathead minnow chronic toxicity test when performed in laboratory facilities other than the ESD Toxicology Laboratory, Room 12, Building 1504.

   a. Follow the procedure described in steps 4.a through 4.e with the following exception:

      1. Screw-cap scintillation vials will be used in lieu of 10-mL beakers for drying and transporting the larvae.

   b. If the facility in which the test is being conducted contains a drying oven, place the vials containing the microweight pans and larvae in the oven for at least 20 minutes at 100 ± 2°C. Remove the vials from the oven and let cool at least 20 minutes before capping and transporting to the ESD Toxicology Laboratory.

   c. Upon return to the ESD Toxicology laboratory, uncap the vials and place in a suitable container. Cover the container with perforated aluminum foil and place in a drying oven for at least two hours at 100 ± 2°C.

   d. Remove the container from the oven and place in a desiccator a minimum of four hours to cool the larvae before weighing them on a calibrated microbalance.

   e. Record the final pan weights and the number of larvae surviving in each beaker on the "Fathead Minnow Weight and Survival Data for Lotus Program" logsheet that was prepared in step 1.b.

   f. If the facility does not contain a drying oven, transfer the larvae to the preweighed microweight pans in the corresponding scintillation vials. If possible, allow the larvae to air dry at least 24-h before capping the vials and transporting them to the ESD Toxicology Laboratory. Continue with the procedure described in steps 5.c through 5.e.

6. Calculate the mean percent survival and mean growth of larvae in each concentration using a Lotus 1-2-3 program.

   a. Important keys and notes about the directions below.

      (1) Use the arrows on the keyboard to move around the worksheet.

      (2) Use the forward slash (/) to get to the main menu.

      (3) Use the Esc key to get out of trouble and move to the previous selection/menu.

      (4) Commands to be typed are in single quotes, do not type the quotation marks.

   b. Turn on the computer and printer if they are not already on.

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

c. Start Lotus 1-2-3.

(1) If the prompt on the screen is C:\, type '123', and press enter;

(2) If the prompt is C:\WP50, type 'cd\', enter, type '123', and press enter;

(3) If the prompt is A:\ type 'C:', press enter, type '123' and press enter;

(4) If the prompt is B:\ type 'C:', press enter, type '123', and press enter.

d. Retrieve the Lotus 1-2-3 worksheet entitled FHM.WK1.

(1) Obtain disk (low density) labeled "FHM Worksheet". It will be next to the computer designated for laboratory use or available from the Principal Investigator.

(2) Place the disk in the lower (B) drive.

(3) Type '/' (forward slash).

(4) Type 'F' (file).

(5) Type 'R' (retrieve).

(6) Use the arrows on the keyboard to highlight FHM.WK1 (If you don't see FHM.WK1 at the top of the screen, press the page down key until you find FHM.WK1)

(7) Press enter.

e. Enter the Test Description Data.

(1) Use the arrows to highlight the entry after "Date:" (column B, row 1). Type an apostrophe ('), the month the test was conducted, a forward slash (/) the first day of the test, a hyphen (-), the last day of the test, a forward slash (/), and the year that the test was conducted. For example: '01/05-12/89. Press enter.

(2) Use the arrows to highlight the entry after "Test Name:" (column B, row 3). Type the name of each test conducted and press enter. For example, Coal Yard Runoff Treatment Facility, Nonradiological Wastewater Treatment Plant.

(3) Use the arrows to highlight the entry after "Test #:" (column B, row 5). Type an apostrophe ('), test number(s), and press enter. For example: '298,299.

(4) Use the arrows to highlight the entry after "Tester:" (column B, row 7). Type your name and press enter.

(5) Use the arrows to highlight the entry after "Comments:" (column B, row 9). If you need to change the comments, type the new information and press enter.

f. Enter the fathead minnow weight and survival data.

(1) Obtain the "Fathead Minnow Weight and Survival Data for Lotus Program" logsheet (exhibit SOP-9.1) that you filled out when you weighed the microweight pans and the microweight pans + larvae.

(2) Use the arrows on the keyboard to highlight the first concentration in the "A" column.

(3) If the concentration needs to be changed (refer to the log sheet), type an apostrophe ('), the new concentration, and press enter. Repeat for each concentration. If you need to add additional concentrations to the worksheet go to step 5g. If there are more concentrations on the worksheet then you need, go to step 5h.

(4) Use the arrows on the keyboard to highlight the first entry under column "C" entitled "PanWt". Refer to the log sheet, type in the new pan weight, and press enter.

(5) Use the right arrow to highlight the first entry under column "D" entitled "Pan+Larv". Refer to the log sheet, type in the new pan+larvae weight, and press enter.

(6) Use the right arrow to highlight the first entry under column "E" entitled "NoSurv". Refer to the log sheet, type in the number of larvae surviving in that replicate, and press enter.

(7) Repeat for each successive replicate and concentration.

(8) DO NOT enter any data in columns "F" to "J".

g. Add extra concentrations to the "FHM.WK1" worksheet as follows.

(1) Determine the number of extra concentrations needed.

(2) Use the arrows to move to the first concentration in the worksheet. (You should be in the "A" column.)

(3) Type '/' (forward slash).

(4) Type 'C' (copy).

(5) Use the right arrow to move the curser over to the "J" column.

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

(6) Use the down arrow to highlight the number of extra concentrations that you need.

(7) Press enter.

(8) Use the down arrow to go to the first blank line at the bottom of the work sheet (this is where the extra concentrations will be added).

(9) Press enter. The extra concentrations should appear at the bottom of the worksheet. Go to step 5f to enter data.

h. Erase extra concentrations as follows.

(1) Place the cursor on "Rep#1" line in "A" column of the first concentrations that you want to erase.

(2) Type '/' (forward slash).

(3) Type 'R' (range).

(4) Type 'E' (erase).

(5) Use the right arrow to move the cursor to the "J" column.

(6) Use the down arrow to highlight the number of concentrations that you want to erase.

(7) Press enter.

i. When you have "0" survival in a replicate complete the following steps.

(1) Use the arrows on the keyboard to move the cursor to the "C" column for that replicate.

(2) Type '/' (forward slash).

(3) Type 'R' (range).

(4) Type 'E' (erase).

(5) Use the right arrow to move the cursor to the "G" column.

(6) Press enter.

(7) Use the left arrow to move the cursor to the "E" column titled "NoSurv".

(8) Type '0'.

(9) Press enter.

j When all the new data have been entered, save the file onto the disk as follows.

(1) Type '/' (forward slash).

(2) Type 'F' (file).

(3) Type 'S' (save).

(4) "Enter save file name: B:\fhm.wk1" will appear at the top of the screen.

(5) Type tst followed by the test number. For example: tst312.

(6) Press enter.

k. Print the worksheet as follows.

(1) Press the "Home" key (this will move you to the top of the worksheet).

(2) Type '/' (forward slash).

(3) Type 'P' (print).

(4) Type 'P' (printer).

(5) Type 'R' (range).

(6) Use the arrows on the keyboard to highlight the entire worksheet.

(7) Press enter.

(8) Type 'O' (options).

(9) Type 'S' (set-up). If the set-up reads "\015" press enter (this compresses the print). If the set-up does not read "\015", enter '\015'.

(10) Type 'Q' (quit).

(11) Type 'A' (align). This tells the printer to align the top of the page. Make sure your paper is lined up in the printer.

(12) Type 'G' (go).

l. Exit the Lotus 1-2-3 program as follows.

(1) Type '/' (forward slash).

(2) Type 'Q' (quit).

(3) Type 'Y' (yes).

7. Test acceptability criteria is based on 80% or greater survival in the controls and a mean dry weight of surviving control larvae of ≥ 0.25 mg/fish. Notify the Group Leader if the acceptability criteria are not achieved.

8. Glue the Lotus 1-2-3 weight and survival worksheet in the registered fathead minnow logbook.

9. The technician responsible for conducting the test will make a Xerox® copy of the applicable test logsheets from the registered fathead minnow logbook. Place the copies in the backup fathead minnow test notebook.

10. File the original "Fathead Minnow Weight and Survival Data for LOTUS Program Logsheet" in the fathead minnow original weight sheet notebook located in Room 12. Building 1504.

Approval

All Standard Operating Procedures generated by, revised by, and/or applicable to the ESD Toxicology Laboratory must receive the signed approval of the Group Leader.

Approved by Lynn A. Kszos — Group Leader — 9/16/93 — Date

Effective Date 10/1/93

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

FATHEAD MINNOW TEST INFORMATION

Test Name: _____ Test No.:_____

Test Conducted From _____ (Day 0) To _____ (Day 7)

Sites/Concentrations Tested: _____

_____

Stock (if applicable):_____

Control Water Type (✓):

20% Dilute Mineral Water (DMW) + Trace Metals ☐
25% Dilute Mineral Water (DMW) + Trace Metals ☐
Other (describe):_____ ☐

Source of Test Larvae (✓):

ESD Cultures ☐
Florida Bioassay ☐
Other (described)_____ ☐

Date larvae hatched:_____ Initials:_____

==========

Record of Minor Test Non-Conformities		
Date	Description of Non-Conformity	Initials

Exhibit SOP-9.1. Fathead Minnow Test Information Logsheet

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Fathead Minnow - Daily Test Information Logsheet

TEST NAME: _____ TEST DATES: _____ TEST NO.: _____

Daily Test Information	Temperature Information			Feeding Information		Test Initiation, Water Change, or Test Termination		Control Water Batch No.
Date and Initials	Water Bath (°C)	Beaker (°C)	Thermo. No.	Fed 100 µl Brine Shrimp (✓)	Feeding Time	Start Time	End Time	additional comments
Day 0:					___ am ___ pm			
Day 1:					___ am ___ pm			
Day 2:					___ am ___ pm			
Day 3:					___ am ___ pm			
Day 4:					___ am ___ pm			
Day 5:					___ am ___ pm			
Day 6:					___ am ___ pm			
Day 7:								

Exhibit SOP-9.2. Fathead Minnow Daily Test Information Logsheet

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SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Fathead Minnow Daily Test Results

Test Name:_____ Test No.:_____ Dates:_____ to _____

Comment Codes: C = Clear; D = Dead; Fg = Fungus; K = Killed by siphoning; M = Missing; Sk = Sick; SM = Unusually small; SOR = Siphoned out and returned; W = Wounded by siphoning

Conc.	REP	Enter the number alive and comment code, if applicable						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	1							
	2							
	3							
	4							
	total alive							
	1							
	2							
	3							
	4							
	total alive							
	1							
	2							
	3							
	4							
	total alive							
	1							
	2							
	3							
	4							
	total alive							

Exhibit SOP-9.3. Fathead Minnow Daily Test Results Logsheet

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Fathead Minnow Shipment Logsheet

Larvae Source:	No. Received (Approx.):	Date Received:	Received By: (Initials)

	Hour							
	Initial	1	2	3	4	5	6	7
Temp. (°C)								
Time:								
Therm. No.								
Initials:								

Comments (e.g., condition of larvae received)

Exhibit SOP-9.4. Fathead Minnow Shipment Logsheet

SUBJECT: FATHEAD MINNOW CHRONIC TOXICITY TEST

Fathead Minnow Weight and Survival Data for LOTUS Program

Test Name:____________________ Test No.:__________

Calibration of Cahn C-30 Microbalance (use 20 mg weight) (acceptable range = 19.9000-20.1000)

1. Weighing empty pans
   Weight Before:__________
   Weight After: __________
2. Weighing pans + larvae
   Weight Before:__________
   Weight After: __________

Site/ Conc.	REP	Pan Wt.	Pan + Larvae	No. Surv	Site/ Conc.	REP	Pan Wt.	Pan + Larvae	No. Surv
Initial	1					1			
	2					2			
	3					3			
	4					4			
Control	1					1			
	2					2			
	3					3			
–	4					4			
	1					1			
	2					2			
	3					3			
	4					4			
	1					1			
	2					2			
	3					3			
	4					4			
	1					1			
	2					2			
	3					3			
	4					4			
	1					1			
	2					2			
	3					3			
	4					4			
	1					1			
	2					2			
	3					3			
	4					4			

Exhibit SOP-9.5. Fathead Minnow Weight and Survival Data for Lotus Program Logsheet

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SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

Purpose

To measure the chronic toxicity of ambient or waste waters to *Ceriodaphnia* in 7-d static renewal exposures.

Reference

EPA Test Method 1002.0, in C. I. Weber et al., *Short-Term Methods for Estimating the Chronic Toxicity of Effluent and Receiving Water to Freshwater Organisms*, EPA/600/4-89/001 (March 1989).

Equipment

Ceriodaphnia neonates (collected per SOP-4)
Temperature-controlled water bath or environmental chamber (maintained at 25 ± 2°C)
Dilute mineral water (DMW; see SOP-1)
1-oz disposable plastic beakers (pre-rinsed in distilled water, if necessary)
8-oz specimen containers
Glass tubing (2-mm ID) cut to approximately 17 cm, with ends polished
Pasteur pipettes
1-mL pipette bulbs
Holding racks for 1-oz plastic cups
Randomizing templates (exhibit SOP-10.1)
Thermometer
Ceriodaphnia food (YCT and *Selenastrum*; see SOP-3)
Volumetric flasks
Graduated cylinders
Fixed- or adjustable-volume pipette (e.g., Eppendorf)
Light table
Binocular stereomicroscope (optional)
Magnifying lamp (optional)
Registered *Ceriodaphnia* toxicity test logbook
Sample randomizing template (exhibit SOP-10.1)
Ceriodaphnia Information Sheet for Randomized Test (exhibit SOP-10.2)
Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test (exhibit SOP-10.3)
Ceriodaphnia Daily Test Information Logsheet (exhibit SOP-10.4)
Ceriodaphnia Information Sheet for Non-Randomized Test (exhibit SOP-10.5)
Daily Survival and Reproduction Logsheet For Non-Randomized *Ceriodaphnia* Test (exhibit SOP-10.6)
Lotus 1-2-3® computer software program
WordPerfect® computer software program
SAS® computer software program
Data disk labeled "*Ceriodaphnia* SAS program"

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

I. Procedure for conducting the *Ceriodaphnia* randomized test

NOTE: Toxicology Laboratory personnel have the option of conducting ambient tests using either this method or the proceeding method.

A. In preparation for the test, complete the following steps.

1. Glue a "*Ceriodaphnia* Information Sheet for Randomized Test", a "Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test", and a "*Ceriodaphnia* Daily Test Information Logsheet" into the registered *Ceriodaphnia* toxicity test logbook. Initial and date each logsheet so that half of the writing extends onto the logbook page.

2. Prepare the glassware. Label the necessary volumetric flasks, graduated cylinders, and 8-oz. specimen containers with the site identification (sample name) and dilution. In addition, label 8-oz. specimen containers with the site identification, dilution, and "final pH and D.O." These containers will be used to collect test water during the daily renewal.

3. Collect the neonates for the toxicity test (see SOP-4).

B. To start the test, the following steps are performed.

1. Prepare the test concentrations specified in the test write-up prepared by the Laboratory Steward or designee in accordance with the procedure described in SOP-8. Include a "control" (consisting DMW) for each individual test conducted.

2. Record the concentrations on the "*Ceriodaphnia* Information Sheet for Randomized Test", reserving "1" for the control (DMW).

3. Record the following additional information on the "*Ceriodaphnia* Information Sheet for Randomized Test."

   a. Test name, test number, test dates, stock used (if applicable), control water (DMW) and dilution water types and batch numbers, the source of test animals, and your initials.

4. Select a randomizing template. Use a different template for each holding rack needed to conduct the test. Record the template number on the "*Ceriodaphnia* Information Sheet for Randomized Test" logsheet.

5. Place the template under the holding rack so that the numbers on the template are aligned with the holes in the rack. Pour 15 mL of each test concentration into ten individual replicate (REP) beakers and place the beakers in the corresponding positions in the rack. For example, the ten beakers containing control water are placed in each of the holes where there is a "1." Continue this procedure for each test concentration.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

6. Using the fixed- or adjustable-volume pipette, add 50 µL of YCT and 50 µL of *Selenastrum* to each beaker.

7. Using a Pasteur pipette, place one neonate (collected as in SOP-4) in each test beaker as follows.

   a. Obtain a beaker with a minimum of 8 neonates from one adult (collected as in SOP-4). Place one neonate in each of the six test beakers in the first column on the test board. Select another beaker with a minimum of 8 neonates from one adult and place one neonate into each of the six test beakers in the second column of the test board. Repeat this process until each of the 60 beakers contains one neonate.

   b. This procedure allows the performance of each adult to be tracked. Adults that produce one weak offspring or male offspring have a greater likelihood of producing more young that are males or weak. In this manner, if all of the REPs for each concentration are male or have low reproduction, they may be excluded from statistical analyses.

8. Record the following information on the "*Ceriodaphnia* Daily Test Information Logsheet." Refer to the logsheet for specific information required.

   a. Test name, test number, test dates, temperature information, feeding information, starting and ending times (i.e., the time the first neonate was added to the test to the time the last neonate was added), DMW batch number, test initiation date (Day 0), and your initials.

9. Record the following information on the "Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test."

   a. Test name, test number, test dates, and template number.

C. Perform a 24-h renewal each day during the 7-d test period.

1. Prepare fresh concentrations each day.

2. Using a polished glass tube affixed to a pipette bulb, transfer each animal to a new test beaker containing 15 mL of freshly prepared test solution and 50 µL of YCT and 50 µL of *Selenastrum*. Count and record (for the appropriate day and replicate) the number of young in each beaker on the "Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test." If no young were produced, code the adult appropriately (refer to the codes on the "Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test"). Holding the beaker over a light table will facilitate counting the number of young produced. A dissecting microscope may also be used.

3. Carefully pour the old test water from at least eight REP beakers into the 8-oz specimen containers marked "final pH and DO." NOTE: you will only need to collect test water from the concentrations used for water chemistry analyses. Discard the remaining neonates produced by the test animals and the old test water in accordance with the procedure outlined in SOP-7.

4. Record the date of transfer, time of transfer, feeding information, DMW batch number, temperature information, and your initials on the "*Ceriodaphnia* Daily Test Information Logsheet" in the registered *Ceriodaphnia* toxicity test logbook.

5. Record minor test non-conformities (e.g., a temperature that falls outside the acceptable range), on the "*Ceriodaphnia* Information Sheet for Randomized Test."

D. Terminating the test involves the following.

1. The test should be terminated when 60% of the surviving females in the control have had their third brood and there is an average of 15 or more neonates per surviving female in the control. The test is terminated ± 2 h from the time it was initiated.

2. Record the number of neonates in each beaker as described in C.2 above.

3. Record the starting and ending times of the test take down on the "*Ceriodaphnia* Daily Test Information Logsheet." Also record the water bath or incubator temperature, beaker temperature, thermometer number, and your initials on the logsheet.

4. Collect the old test water for final pH and D.O. measurements as described in C.3. Discard the remaining test water, neonates and adults in accordance with the procedures outlined in SOP-7.

E. Generate the summary statistics and tables using SAS© and WordPerfect© computer software programs.

1. Create a data file using WordPerfect.

a. Turn on the computer and printer if they are not already on.

b. Insert a data disk into a disk drive.

c. Access the WordPerfect® program. Commands to be typed are in single quotes; do not type the quotation marks.

(1) If the prompt on the screen is C:\>, type 'cd WP51' and press enter. Type 'WP' and press enter again.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

(2) If the prompt on the screen is A:\> or B:\>, type 'C:' and press enter, then type 'cd WP51' and press enter again. Type 'WP' and press enter.

d. Important notes about entering data for the data file.

(1) Capitalize all letters.

(2) Enter the reproduction data for each test beaker starting with the beaker number. Use two characters to enter the beaker number (e.g., 03, 27).
On the same line enter the reproduction data. Space between each entry and return after each line. EXCEPTION: DO NOT RETURN AFTER THE LAST LINE IS ENTERED. This will normally be line 60.

Example: 34 0 0 4 0 0 18 22 (Test beaker number 34; followed by seven days of reproduction data.)

(3) Enter a zero (0) if the animal was alive and no reproduction was observed for the test day.

(4) If the test was not conducted for all seven days, type a period (.) in place of the missing reproduction data.

Example: 27 0 0 5 0 15 19 . (Test beaker number 27. The test was terminated on day 6. No reproduction data for day 7 were available.)

(5) If the test animal reproduced and died, enter the number of neonates followed by a capital "X." Type an X for each remaining day the test was conducted.

Example: 03 0 0 8X X X X . (Test beaker number 3. The adult died on day 3 and had 8 young. Test was terminated on day 6.)

(6) If the test animal died and did not reproduce, enter a capital "X". Type an X for each remaining day the test was conducted.

Example: 49 0 0 X X X X X (Test beaker number 49; animal died on day 3.)

(7) If the test animal was male, enter a capital "M" for each day the test was conducted.

Example: 17 M M M M M M M

(8) If the test contained less than 60 test beakers, enter only the beaker numbers and reproduction data that were used.

e. Enter the *Ceriodaphnia* survival and reproduction data.

f. When all the data are entered, convert the data file to an ASCII file.

(1) Press Ctrl + F5.

(2) Select option 1 (DOS Text).

(3) Select option 1 (SAVE) and name the ASCII file.

(a) Suggestion: Use the test name, test number, or a combination thereof, followed by an ASC extension. Also, designate the disk drive. For example, if your data disk is in the "A" drive, type 'A:\WOC932.ASC' and press enter.

g. Print the data file.

(1) Press shift + F7.

(2) Select option 1 (Full Document)

h. Verify data input.

(1) On the copy of the data file you printed in step g, write the test name, test number, test date, and template number. Verify the accuracy of the inputted data against the original data in the registered *Ceriodaphnia* test logbook. Initial and date the printed copy after the data have been verified. File the original in the "*Ceriodaphnia* toxicity test ASCII files" notebook located in Building 1504, Room 12.

g. Exit WordPerfect.

(1) Press F7.

(2) Press 'Y' if you want to save the data file you created. It is not necessary to save this file in WordPerfect since it was saved as an ASCII file. If you do save the data file, follow the instructions described in 3a above except do not use the ASC extension.

(3) Press 'N' if you do not want to save the data file, then press 'Y' to exit WordPerfect.

2. Generate the summary statistics and tables using the SAS® computer program.

a. Access the SAS® program. Commands to be typed are in single quotes; do not type the quotation marks.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

(1) If the prompt on the screen is C:\>WP51, type 'cd C:\' and press enter.

(2) If the prompt on the screen is C:\>, type 'cd SAS' and return. When C:\>SAS> appears, type 'SAS' and return again.

(3) If the prompt on the screen is A:\> or B:\>, type 'C:' and press enter. Follow the instructions described above.

b. Insert the data disk titled "*Ceriodaphnia* SAS Program" into a disk drive. NOTE: All proceeding instructions will be based on using the B drive for the data disk. If another drive is used, make the appropriate substitution.

c. Important keys and notes about using the SAS program.

(1) The SAS screen is divided into three sections: Output, Log, and Program Editor. Each section title is followed by a command line.

(2) Press F5 to move between the sections.

(3) Press F7 to pull up a full screen for the section in which the curser lies.

(4) Press F10 to execute the SAS program.

(5) Ctrl + break ("pause key") will stop the program.

d. Load the SAS program written for compiling the *Ceriodaphnia* reproduction data. Commands to be typed are in boldface print.

(1) If the curser is not on the command line of the Program Editor section, press F5 until it is in the proper location.

(2) At the command line, type **include "b:\table_prg"** and press enter.

(3) Press F7 to pull up a full screen.

(4) Use the page down (PgDn) and/or arrow keys (↓ ↑) to move to the last line (should be line 271). The line should read as follows:

%cerio(title,tempfil,datafil,temp,nconc,days,c1,c2,c3,c4,c5,c6)

(5) Replace the following. Text to be typed are in boldface print. Do not space after each comma.

Title — Designate the name. Suggestion: use the test name or test number. Example: K25STP.940 = K-25 Sewage Treatment Plant, test number 940.

Tempfil — b:\template.dat

Datafil — Type the name of the ASCII file you created using WordPerfect. Designate the disk drive. Example: b:\K25STP.ASC

Temp — Enter the template number.

Nconc — Enter 6.

Days — Enter the number of days the test ran. This will normally be 6 or 7.

C1-C6 — Enter the concentrations used in order from 1-6. Refer to the "*Ceriodaphnia* Information Sheet for Randomized Test" in the registered *Ceriodaphnia* logbook. Since C1 is reserved for the control, this must be zero (0). Enter the concentrations tested for C2-C6 as whole numbers. DO NOT ENTER DECIMAL POINTS OR PERCENT SIGNS. If there were less than six total concentrations tested (including the control), use zero (0) as a place-holder for the missing concentrations. THERE MUST BE SIX TOTAL ENTRIES. Example: 0, 100, 50, 25, 12, 0 (The first zero designates the control, the last zero is a place-holder).

(6) Press F10 to run the SAS program.

(7) After the program has finished compiling the data, press F5 once to move the curser to the Output section on the screen.

(8) Type file 'LPT1:' or file 'prn' to send the output to the printer. Note: This command is appropriate for the computer and printer located in Room 12.

(9) After the document has printed, type bye to exit SAS.

(10) Glue a copy of the output document in the registered *Ceriodaphnia* toxicity test logbook. Initial and date each sheet so that half of the writing extends onto the logbook page.

(11) The technician responsible for conducting the test will make a Xerox® copy of the applicable log sheets from the registered *Ceriodaphnia* test logbook. Place the copy in the backup *Ceriodaphnia* test notebook.

3. Test acceptability criteria is based on 80% or greater survival in the controls and reproduction in the controls must average 15 or more young per surviving female. If the test does not meet these criteria, notify the Group Leader or designee.

II. Procedure for conducting the non-randomized *Ceriodaphnia* test

A. In preparation for the test, complete the following steps.

1. Prepare the glassware. Label 1-oz plastic beakers with the sample identification, dilution, and replicate (REP) number (ten REPs are typically used per concentration or site; additional REPs may be required for some tests). Label the necessary volumetric flasks, graduated cylinders, and beakers.

2. Collect neonates for the toxicity test (step B, SOP-4).

3. Glue a "*Ceriodaphnia* Information Sheet for Non-Randomized Test" (exhibit SOP-10.4), a "*Ceriodaphnia* Daily Test Information Logsheet" (exhibit SOP-10.3), and a "Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test" (exhibit SOP-10.5) into the registered *Ceriodaphnia* toxicity test logbook. Note: You may need more than one daily survival and reproduction log sheet for the test. Initial and date each sheet so that half of the writing is on the log sheet and half extends onto the logbook page.

B. To start the test, the following steps are performed.

1. Prepare the test concentrations specified in the test write-up in accordance with the procedure described in SOP-8. Include a "control" (consisting of DMW) for each individual test conducted. Record the sites/concentrations tested on the *Ceriodaphnia* Information Sheet for Non-Randomized Test" logsheet.

2. Record the following additional information on the "*Ceriodaphnia* Information Sheet Non-Randomized Test" logsheet.

   a. Test name, test number, test dates, stock used (if applicable), control water and dilution water types and batch numbers, the source of test animals, and your initials.

3. Pour 15 mL of test solution in each of the ten REP beakers.

4. Using a fixed- or adjustable-volume pipette, add 50 µL of YCT and 50 µL of *Selenastrum* to each beaker.

5. Place one neonate (collected as in SOP-4) in each test beaker using a Pasteur pipette as follows.

   a. Obtain a beaker with a minimum of 8 neonates from one adult (collected as in SOP-4). Place one neonate in each of the six test beakers in the first column on the test board. Select another beaker with a minimum of 8 neonates from one adult and place one neonate into each of the six test beakers in the second column of the test board. Repeat this process until each of the 60 beakers contains one neonate.

   b. This procedure allows the performance of each adult to be tracked. Adults that produce one weak offspring or male offspring have a greater likelihood of producing more young that are males or weak. In this manner, if all of the REPs for each concentration are male or have low reproduction, they may be excluded from statistical analyses.

6. Place the beakers in holding racks, and put the racks in the temperature-controlled water bath or environmental chamber.

7. Record the following information on the "*Ceriodaphnia* Daily Test Information Logsheet." Refer to the logsheet for specific information required.

   a. Test name, test number, test dates, temperature information, feeding information, starting and ending times (i.e., the time the first neonate was added to the test to the time the last neonate was added), DMW batch number, test initiation date (Day 0), and your initials.

8. Record the following information on the "Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test."

   a. Test name, test number, and test dates.

C. Perform a 24-h renewal each day during the 7-d test period.

1. Prepare fresh dilutions each day.

2. Using a polished glass tube affixed with a pipette bulb and a dissecting microscope, if necessary, transfer each animal daily to a new test beaker containing 15 mL of freshly prepared test solution and 50 µL of YCT and 50 µL of *Selenastrum*. Count and record (for the appropriate day and replicated number) the number of young in each beaker on the "Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test" in the registered *Ceriodaphnia* logbook. Holding the beaker over a light table will facilitate counting the number of young produced.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

3. If no young were produced, if the animal died, or if the animal is male, use the appropriate code and record on the "Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test." Refer to the logsheet for the codes.

4. After counting, discard the neonates produced, any dead animals, and the old test solution in accordance with the procedure described in SOP-7.

5. Record the date of transfer, time of transfer, feeding information, temperature information and your initials on the "*Ceriodaphnia* Daily Test Information Logsheet" in the registered *Ceriodaphnia* logbook.

6. Record minor test non-conformities (e.g., a temperature that falls outside the acceptable range), on the "*Ceriodaphnia* Information Sheet for Randomized Test."

D. Terminating the test involves the following.

1. The test should be terminated when 60% of the surviving females in the control have had their third brood and there is an average of 15 or more neonates per surviving female in the control. The test is terminated $\pm$ 2 h from the time it was initiated.

2. Count and record the number of neonates in each beaker as described in C.2 through C.3.

3. Record the starting and ending times of the test take down on the "*Ceriodaphnia* Daily Test Information Logsheet."

4. Discard the test animals, neonates, and test solutions per the procedure described in SOP-7.

5. Record the date the test was terminated, temperature information, and your initials on the "*Ceriodaphnia* Daily Test Information Logsheet" in the registered *Ceriodaphnia* logbook.

E. Calculate the total number of offspring produced, the mean number of offspring for all females, and the mean number of offspring for surviving females, using the Lotus 1-2-3® computer program.

1. Important keys and notes about using the Lotus 1-2-3 computer program.

(a) Use the arrows on the keyboard to move around the worksheet.

(b) Use the forward slash (/) to get to the main menu.

(c) Use the Esc key to get out of trouble and move to the previous selection/menu.

(d) Commands to be typed are in single quotes; do not type the quotation marks.

(e) The Lotus 1-2-3 worksheet is divided into two parts. On the left (Columns A-K) there are two rows of squares that will do the statistical calculations. This is the part you will print out. On the right there are rows of squares into which you will enter the test data. The information on the right will not be printed.

(f) There are numbers in the upper right hand corners of the boxes on the left side of the worksheet under column "E" or "J". These numbers correspond to the numbers above each "Concentration/Site" on the right side of the worksheet under column "L".

2. Turn on the computer and printer if they are not already on.

3. Start Lotus 1-2-3.

(a) If the prompt on the screen is C:\, type '123', and press enter;

(b) If the prompt is C:\WP50, type 'cd\', press enter, type '123', and press enter;

(c) If the prompt is A:\ type 'C:', press enter, type '123', and press enter;

(d) If the prompt is B:\ type 'C:', press enter, type '123', and press enter.

4. Retrieve the Lotus 1-2-3 worksheet entitled CDBLANK1.WK1.

(a) Obtain the disk (low density) labeled "CDBLANK". It will be next to the computer designated for laboratory use or available from the Principal Investigator.

(b) Place the disk in the lower (B) drive.

(c) Type '/' (forward slash).

(d) Type 'F' (file).

(e) Type 'R' (retrieve).

(f) Use the arrows on the keyboard to highlight CDBLANK1.WK1 (If you don't see CDBLANK1.WK1 at the top of the screen, press the page down key until you find CDBLANK.WK1).

(g) Press enter.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

5. Enter the Test Description Data.

   (a) Use the arrows to highlight the entry after "TEST NAME:" (column C, row 3). Type in the name of the test and press enter. For example: Coal Yard Runoff Treatment Facility.

   (b) Use arrows to highlight the entry after "TEST DATE:" (column E, row 3). Type an apostrophe ('), the month the test was conducted, a forward slash (/) the first day of the test, a hyphen (-), the last day of the test, a forward slash (/), and the year that the test was conducted. For example: '01/05-12/89. Press enter.

   (c) Use the arrows to highlight the entry after "TEST NUMBER:" (column H, row 3). Type an apostrophe ('), the number(s) of the test(s) and press enter. For example: '554.

   (d) Use the arrows to highlight the entry after "PERSONNEL:" (column J, row 3). Type your name and press enter.

6. Enter *Ceriodaphnia* survival and reproduction data. (Note: The steps below this point may be completed for up to ten concentrations/sites. The worksheet is so large that more than ten concentrations/sites becomes cumbersome. If you have more than ten concentrations/sites, complete all of the steps below for the first ten concentrations/sites, call up the CDBLANK2.WK1 worksheet, and repeat all steps for the remaining concentrations/sites.)

   (a) Obtain the registered *Ceriodaphnia* logbook which contains the test data.

   (b) Enter the test concentrations/sites as follows.

      (1) Use the arrows to move to the "L" column and highlight the first concentration.

      (2) Type an apostrophe (') and the test concentration/site.

      (3) Repeat for each test concentration/site (up to a total of ten; see note at step f.)

   (c) Enter the survival data for each replicate of each concentration/site as follows.

      (1) Use the right arrow to move to the "P" column. Highlight the first replicate under the first concentration/site.

      (2) If the animal that represents that replicate (refer to the registered *Ceriodaphnia* logbook) was female and survived, enter a '1' in that cell.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

(3) If the animal that represents that replicate was a female and died during the test or was a male, enter a '0' in that cell.

(4) Repeat for each replicate of each concentration/site.

(d) Enter the reproduction data for each replicate of each concentration/site as follows.

(1) Use the arrows to move to the "R" column. Highlight the first replicate under the first concentration/site.

(2) If the animal that represents that replicate did not have any young enter a '0'.

(3) If animal that represents that replicate had young, or died and had young, enter the number of young the animal produced.

(4) If the animal was male, enter a '0'.

(5) Repeat for each replicate.

(e) Do not enter anything in column "T".

(f) If you entered a '0' in column "P" for any replicate (step f(4)), complete the following.

(1) Use the arrows to move to column "V" with the heading "Corrected # Young SF" (short for surviving females).

(2) Use the arrows to highlight the word "blank".

(3) Type '/' (forward slash).

(4) Type 'R' (range).

(5) Type 'E' (erase).

(6) Press enter (the word "blank" should be erased).

(g) Enter the concentration/site names into the statistical part of the worksheet (left side) as follows.

(1) Use the arrows to highlight the cell to the right of the first "Concentration/Site:" (either column "D" or column "I").

(2) Type the concentration/site that corresponds to the same numbered box on the right hand of the worksheet. For example: if box #5 on the right hand side of the worksheet is labeled "Site 3", label box #5 on the left hand of the worksheet "Site 3".

(3) Repeat for each concentration/site.

7. When all the new data have been entered, save the file onto the disk as follows.

(a) Type '/' (forward slash).

(b) Type 'F' (file).

(c) Type 'S' (save).

(d) "Enter save file name: B:\CDBLANK1.WK1" will appear at the top of the screen.

(e) Type cd followed by the test number. For example: cd312.

(f) Press enter.

8. Print the worksheet as follows.

(a) Press the "Home" key (this will move you to the top of the worksheet).

(b) Type '/' (forward slash).

(c) Type 'P' (print).

(d) Type 'P' (printer).

(e) Type 'R' (range). The top of the screen should now read "range A1..K86". If the top of the screen does not read "A1..K86", press Esc, press home to get to A1, type a period (.), and highlight all of the boxes from A1 through K86.

(f) Press enter.

(g) Type 'O' (options).

(h) Type 'S' (set-up). If the set-up reads "\027\048\015" press enter (this compresses the print). If the set-up does not read "\027\048\015", enter '027\048\015'.

(i) Press enter.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

(j) Type 'Q' (quit).

(k) Type 'A' (align). This tells the printer to align the top of the page. Make sure your paper is lined up in the printer.

(l) Type 'G' (go).

9. Exit the Lotus 1-2-3 program as follows.

(a) Type '/' (forward slash).

(b) Type 'Q' (quit).

(c) Type 'Y' (yes).

10. Make a Xerox® copy of the Lotus worksheet. Glue the original in the registered *Ceriodaphnia* logbook.

11. The technician responsible for conducting the test will make a Xerox® copy of the applicable test logsheets from the registered *Ceriodaphnia* test logbook. Place the copies in the backup *Ceriodaphnia* test notebook.

12. Test acceptability criteria is based on 80% or greater survival in the controls and reproduction in the controls must average 15 or more young per surviving female. If the test does not meet these criteria, notify the Group Leader or designee.

Approval

All Standard Operating Procedures generated by, revised by, and/or applicable to the ESD Toxicology Laboratory must receive the signed approval of the Group Leader.

Approved by Lynn A. Kaya — Group Manager — 9/13/93 — Date

Effective Date 10/1/93

5	3	3	4	2	4	4	2	6	1
1	5	6	2	5	6	2	5	4	6
2	6	4	6	3	3	1	6	5	4
4	4	2	1	1	5	6	3	2	2
6	1	5	3	4	1	3	1	1	5
3	2	1	5	6	2	5	4	3	3

Exhibit SOP-10.1. Sample randomizing template.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

CERIODAPHNIA INFORMATION SHEET FOR RANDOMIZED TEST

Test Name: _____ Test No.: _____

Test Conducted From _____ (Day 0) To _____ (Day 7)

Sites/Concentrations: 1 = _____ 4 = _____ Template No.:_____
2 = _____ 5 = _____
3 = _____ 6 = _____

Stock (if applicable):_____

Control Water Type (✓):
20% Dilute Mineral Water (DMW) + Trace Metals ☐
25% Dilute Mineral Water (DMW) + Trace Metals ☐
Other (describe):_____ ☐

Dilution Water Type (✓):
20% Dilute Mineral Water (DMW) + Trace Metals ☐
25% Dilute Mineral Water (DMW) + Trace Metals ☐
Other (describe):_____ ☐

Source of Test Animals:

Transferred ESD culture board nos. _____ - _____ on _____ (Wed. date)
from _____ to _____ (time) Initials:_____

Isolated neonates for test on _____ (date) from boards _____
from _____ to _____ (time) Initials:_____

Record of Minor Test Non-Conformities		
Date	Description of Non-Conformity	Initials

Exhibit SOP-10.2. *Ceriodaphnia* Information Sheet for Randomized Test.

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SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test Template No.___

Date:		Test:				Test No.	
Codes: (-)Alive and No Reproduction; (M)Alive and Reproduction; (±)Dead; (±M)Dead and Reproduction; (M)Male							
	Test Beaker Number						
Day	1	2	3	4	5	6	
1							
2							
3							
4							
5							
6							
7							
	7	8	9	10	11	12	
1							
2							
3							
4							
5							
6							
7							
	13	14	15	16	17	18	
1							
2							
3							
4							
5							
6							
7							
	19	20	21	22	23	24	
1							
2							
3							
4							
5							
6							
7							
	25	26	27	28	29	30	
1							
2							
3							
4							
5							
6							
7							

Exhibit SOP-10.3.1. Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test.

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

Daily Survival and Reproduction Log Sheet for Randomized *Ceriodaphnia* Test (cont.)

Date: | Test: | Test No.

Codes: (-)Alive and No Reproduction; (+)Alive and Reproduction; (x)Dead; (x+)Dead and Reproduction; (M)Male

Test Beaker Number						
Day	31	32	33	34	35	36
1						
2						
3						
4						
5						
6						
7						
	37	38	39	40	41	42
1						
2						
3						
4						
5						
6						
7						
	43	44	45	46	47	48
1						
2						
3						
4						
5						
6						
7						
	49	50	51	52	53	54
1						
2						
3						
4						
5						
6						
7						
	55	56	57	58	59	60
1						
2						
3						
4						
5						
6						
7						

Exhibit SOP-10.3.2. Daily Survival and Reproduction Logsheet for Randomized *Ceriodaphnia* Test (cont.)

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

Ceriodaphnia - Daily Test Information Logsheet

TEST NAME: _____ TEST DATES: _____ TEST NO.: _____

Daily Test Information	Temperature Information			Feeding Information				Test Initiation, Water Change or Test Termination		
Date and Initials	Chamber (°C)	Beaker (°C)	Therm. No.	YCT Fed 50 mL	YCT Date	*Selenastrum* Fed 50 mL	*Sel.* Date	Start Time	End Time	
Day 0:										
Day 1:										
Day 2:										
Day 3:										
Day 4:										
Day 5:										
Day 6:										
Day 7:										

Exhibit SOP-10.4. *Ceriodaphnia* Daily Test Information Logsheet

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

CERIODAPHNIA INFORMATION SHEET FOR NON-RANDOMIZED TEST

Test Name:_____ Test No.:_____

Test Conducted From _____ (Day 0) To _____ (Day 7)

Sites/Concentrations Tested:_____

_____

Stock (if applicable):_____

Control Water Type (✓):

20% Dilute Mineral Water (DMW) + Trace Metals ☐
25% Dilute Mineral Water (DMW) + Trace Metals ☐
Other (describe):_____ ☐

Dilution Water Type (✓):

20% Dilute Mineral Water (DMW) + Trace Metals ☐
25% Dilute Mineral Water (DMW) + Trace Metals ☐
Other (describe):_____ ☐

Source of Test Animals:

Transferred ESD culture board nos._____ - _____ on _____(Wed. date)
from _____ to _____ (time) Initials:_____

Isolated neonates for test on _____(date) from boards _____
from _____ to _____ (time) Initials:_____

Record of Minor Test Non-Conformities		
Date	Description of Non-Conformity	Initials

Exhibit SOP-10.5. *Ceriodaphnia* Information Sheet for Non-Randomized Test

SUBJECT: *CERIODAPHNIA* CHRONIC TOXICITY TEST

Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test

DATE: TEST: TEST NO:

CODES: (+) Alive and no reproduction; (+#) Alive and reproduction; (X) Dead; (X#) Dead and reproduction; (M) Male; (DY#) Dead Young

Conc.	Day	REPLICATE 1	2	3	4	5	6	7	8	9	10	Live Adults	No. Young
	1												
	2												
	3												
	4												
	5												
	6												
	7												
	Total												
	1												
	2												
	3												
	4												
	5												
	6												
	7												
	Total												
	1												
	2												
	3												
	4												
	5												
	6												
	7												
	Total												
	1												
	2												
	3												
	4												
	5												
	6												
	7												
	Total												

Exhibit SOP-10.6. Daily Survival and Reproduction Logsheet for Non-Randomized *Ceriodaphnia* Test

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SUBJECT: *CERIODAPHNIA* ACUTE TOXICITY TEST

Purpose

To measure the acute toxicity of water samples to *Ceriodaphnia* during 24-h, 48-h or 96-h exposures.

References

EPA Test Method, in C. I. Weber et al., *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, EPA/600/4-90/027 (September 1991)

Equipment

Ceriodaphnia neonates (see SOP-4)
Temperature-controlled water bath or environmental chamber
Dilute mineral water (DMW; see SOP-1)
Ceriodaphnia food (YCT and *Selenastrum*; see SOP-3)
1-oz. disposable plastic beakers (pre-rinsed in distilled water, if necessary)
8-oz. specimen containers
Glass tubing (2-mm ID) cut to approximately 17 cm, with ends polished
1-mL pipette bulbs
Disposable Pasteur pipettes
Fixed- or adjustable-volume pipettor (e.g. Eppendorf)
Holding racks for 1-oz. plastic beakers
Randomizing templates
Thermometer
Volumetric flasks
Graduated cylinders
Light table
Magnifying lamp or dissecting microscope (optional)
Registered *Ceriodaphnia* toxicity test logbook
Ceriodaphnia Acute Toxicity Test Information Sheet (exhibit SOP-18.1)
Ceriodaphnia Acute Toxicity Test Survival Logsheet (exhibit SOP-18.2)

Procedure

1. In preparation for the test, complete the following steps.

   a. Prepare the glassware. Label the necessary volumetric flasks and graduated cylinders with the sample identification and concentration.

   b. Label 8-oz. specimen containers with the sample identification, concentration, and "final pH and D.O." These containers will be used to collect the test solution during test renewal, if applicable, and at test termination.

c. Collect the neonates for the toxicity test per the procedures described in SOP-4. Neonates must be less than 24 h old.

d. Feed the neonates 50 µL of YCT and 50 µL of *Selenastrum* a minimum of 2 h before their use in a test. Record the time the neonates were fed, the YCT and *Selenastrum* dates, and your initials on the "*Ceriodaphnia* Acute Toxicity Test Information Sheet."

2. To start the test, do the following. NOTE: The proceeding instructions are applicable for the 24-, 48-, and 96-h acute toxicity tests.

a. Glue a "*Ceriodaphnia* Acute Toxicity Test Information Sheet" and a "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet" into the registered *Ceriodaphnia* toxicity test logbook. Initial and date each logsheet so that half of the writing extends onto the logbook page.

b. Record the date the test starts and ends, the test name, and the test number on each of the logsheets and in the index in the registered *Ceriodaphnia* test logbook.

c. Prepare the test concentrations, if necessary. Record the concentrations on the "*Ceriodaphnia* Acute Toxicity Test Information Sheet," reserving "1" for the control. Also record the control water and dilution water types and batch numbers on the logsheet. Note: Some tests may require an in-stream sample be used as the dilution water.

d. Select a randomizing template. Use a different template for each holding rack needed to conduct the test. Record the template number(s) on the "*Ceriodaphnia* Acute Toxicity Test Information Sheet."

e. Position the template underneath the holding rack so that the numbers on the template are aligned with the holes in the rack.

f. Pour a minimum of 15 mL of test solution into each of four replicate (REP) 1-oz disposal beakers. Place the beakers in the corresponding positions in the rack. Note: Each randomizing template has ten positions for each number (1-6) on the template. This test will use four of the ten positions. For example, place each control beaker into a "1" position. If the test is to run 96-h, or if a test solution renewal is required, record the test beaker number (derived from the holding rack) on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet." Repeat this procedure for each test concentration.

g. Using a Pasteur pipette, place six neonates into each test beaker. Keep the amount of water transferred with the neonates to a minimum to avoid dilution of the test solution. Record the time the first neonate was added ("Start Time") to the time the last neonate was added ("Stop Time") on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet."

h. Record the beaker temperature, water bath or environmental chamber temperature, and thermometer number on the "*Ceriodaphnia* Acute Toxicity Test Survival LogsSheet."

i. Put the holding racks containing the beakers into the temperature-controlled (25 ± 1°C) water bath or environmental chamber.

j. If necessary, adjust the photoperiod to 16 h light: 8 h dark.

3. Repeat the following process every day, starting 24-h after the test was begun and continuing until the test is terminated.

a. Remove the holding rack(s) from the water bath or chamber.

b. Record the water bath or chamber temperature, beakers temperature, and the thermometer number on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet."

c. Place the holding rack on a light table for ease in viewing the test animals.

d. Count and record (in the appropriate column) the number of *Ceriodaphnia* surviving in each REP beaker on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet" for the appropriate concentration or site and replicate number. Record comments codes, if any.

e. Record the time the first beaker was counted ("Start Time") to the time the last beaker was counted ("Stop Time") on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet."

f. Record minor test non-conformities (e.g., a temperature that falls outside the acceptable range), on the "*Ceriodaphnia* Acute Toxicity Test Information Sheet."

4. Perform a test renewal at 48 h. This procedure is applicable to 96-h tests only, unless otherwise specified.

a. Approximately two hours before test solution renewal at 48 h, add 50 µL YCT and 50 µL of *Selenastrum* to each test beaker. Record the feeding time and date, the YCT and *Selenastrum* dates, and your initials on the "*Ceriodaphnia* Acute Toxicity Test Information Sheet" in the registered *Ceriodaphnia* toxicity test logbook.

b. Prepare fresh test solutions.

c. Count and record the number of *Ceriodaphnia* surviving in each beaker per the procedure described in steps 3a through 3e.

d. Using a polished glass tube affixed to a pipette bulb and a microscope, if necessary, transfer the surviving test animals in each test beaker to a new beaker containing a minimum of 15 mL of freshly prepared test solution. Holding the beaker over a light table will facilitate the transfer and counting process. Return the beaker containing the test animals to the appropriate location in the holding rack. Refer to the beaker number recorded on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet."

SUBJECT: *CERIODAPHNIA* ACUTE TOXICITY TEST

e. Pour the test solution from each of the four "old" beakers into an 8-oz. specimen container labelled "final pH and D.O." This solution will be used to determine final pH and dissolved oxygen (D.O.) concentration.

f. Discard the test solution per the procedure described in SOP-7.

5. Terminate the test after the neonates have been exposed to the test solution for the required time (24-, 48-, or 96-h).

a. Count and record the number of *Ceriodaphnia* surviving in each beaker per the procedure described in steps 3a through 3e.

b. Calculate and record the total number *Ceriodaphnia* surviving in each concentration or site on the "*Ceriodaphnia* Acute Toxicity Test Survival Logsheet."

c. Carefully pour the test solution from each of the four replicate beakers into an 8-oz. specimen container labelled "final pH and D.O."

d. Discard the test solution per the procedure described in SOP-7.

6. Test acceptability criterion is based on 90% or greater survival in the controls. If survival is <90%, notify the Group Leader for guidance.

7. Make a Xerox® copy of the applicable log sheets and place the copy in the backup *Ceriodaphnia* toxicity test notebook.

Approval

All Standard Operating Procedures generated by, revised by, and/or applicable to the ESD Toxicology Laboratory must receive the signed approval of the Group Leader.

Approved by Lynn A. Kszos
Group Leader
9/14/93
Date

Effective Date 10/1/93

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SUBJECT: *CERIODAPHNIA* ACUTE TOXICITY TEST

Ceriodaphnia Acute Toxicity Test Information Sheet

Test Name:_____ Test No.:_____

Test Conducted From _____ (Day 0) To _____

Test Concentrations: 1 = _____ 4 = _____ Template No.:_____
2 = _____ 5 = _____
3 = _____ 6 = _____

Control Water Type (✓):
20% Dilute Mineral Water (DMW) – Trace Metals Batch No.:_____ ☐
25% Dilute Mineral Water (DMW) – Trace Metals Batch No.:_____ ☐
Other (describe)_____ Batch No.:_____ ☐

Dilution Water Type (✓):
20% Dilute Mineral Water (DMW) – Trace Metals Batch No.:_____ ☐
25% Dilute Mineral Water (DMW) – Trace Metals Batch No.:_____ ☐
Other (describe)_____ Batch No.:_____ ☐

Stock: _____ DMW Batch No.:_____

Source and Age of Neonates:
ESD cultures, Board Nos.:_____ Age:_____

DAY 0 INFORMATION:
Fed each test beaker 50 µL of YCT + 50 µL of *Selenastrum* at _____ (time) on _____ (date)
YCT Date:_____ *Selenastrum* Date:_____
NOTE: Feed neonates ~2 h prior to test initiation. Initials:_____

96-H TEST INFORMATION:
Fed each test beaker 50 µL of YCT + 50 µL of *Selenastrum* at _____ (time) on _____ (date)
YCT Date:_____ *Selenastrum* Date:_____
NOTE: Feed neonates ~2 h prior to test renewal at 48-h.
Transferred test from _____ to _____ (time) on _____ (date)
Initials:_____

Record of Minor Test Non-Conformities		
Date	Description of Non-Conformity	Initials

Exhibit SOP-18.1. *Ceriodaphnia* Acute Toxicity Test Information Sheet

SUBJECT: *CERIODAPHNIA* ACUTE TOXICITY TEST

Ceriodaphnia Acute Toxicity Test Survival Logsheet

Test Name: _____ Test No: _____

Test Conducted From: _____ (day 0) To: _____

Comment Code: M = Missing

		Initials:						
		Start Time:						
		Stop Time:						
		Water Bath/Chamber Temperature (°C):						
		Test Beaker Temperature (°C):						
		Thermometer Number:						
			Enter Number Alive (Enter comment code, if applicable)					
Conc./Site	REP	Beaker No.	Initial No.	24 hr	48 hr	72 hr	96 hr	Total Alive
	1		6					
	2		6					
	3		6					
	4		6					____
	1		6					
	2		6					
	3		6					
	4		6					
	1		6					
	2		6					
	3		6					
	4		6					____
	1		6					
	2		6					
	3		6					
	4		6					____
	1		6					
	2		6					
	3		6					
	4		6					____
	1		6					
	2		6					
	3		6					
	4		6					____

Exhibit SOP-18.2. *Ceriodaphnia* Acute Toxicity Test Survival Logsheet

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SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

Purpose

To determine the relative toxicity of whole sediments to *Hyalella azteca* during a 10-day static-renewal exposure.

Reference

ASTM (American Society for Testing and Materials) 1991. *Standard Guide for Conducting Sediment Toxicity Tests Freshwater Invertebrates*, ASTM E-1383-90, or most recent edition.

Equipment

Hyalella azteca young (0.300 to 0.425 mm size; ~1-2 week old; see SOP-25)
Crystallizing dishes, 250-mL capacity ("counting dishes")
Plexiglass sheets (~1x2 ft.)
30% dilute mineral water (control water; see SOP-1)
Distilled water
Test sediment
Hyalella food (see SOP-24)
Temperature-controlled water bath or environmental chamber (24 ± 2°C)
Dissecting microscope (if necessary)
Fume hood
Disposable Nitrile® gloves
4-L plastic tanks
Plastic or hard rubber spatulas
Disposable syringes (10- and 60-cc) (e.g., B-D Plastipak)
Modified 60-cc syringes (syringe tip is fitted with a ¼" O.D. plastic tubing inside a ¼" ID plastic cylinder cut to ~1" to which a piece of 100-μm Nitex® mesh is attached to one end)
Thermometer
Wash bottles
Stainless steel sieves, 5-in. diameter (#20 ASTM, 0.850 mm, #40 ASTM, 0.425 mm and #60 ASTM, 0.250 mm)
20-L plastic carboy or bucket
Large, wide-mouth plastic funnels
8-oz. specimen containers
1-oz. disposable plastic beakers (pre-rinsed in distilled water, if necessary)
Pasteur pipettes, long (8")
Graduated cylinders
Fixed- or adjustable-volume pipette (e.g., Eppendorf)
Small glass petri dish or equivalent
Hyalella azteca Whole Sediment Toxicity Test - Test Information Sheet (exhibit SOP-21.1)
Hyalella azteca Whole Sediment Toxicity Test - Daily Test Information Logsheet (exhibit SOP-21.2)
Hyalella azteca Whole Sediment Toxicity Test - Test Survival Logsheet (exhibit SOP-21.3)

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

Registered *Hyalella* toxicity test logbook
Hyalella Whole Sediment Toxicity Test - Daily Chemistry Data Logsheet (exhibit SOP-21.4)
Registered Chemistry Logbook (if appropriate)

Procedure

1. Prepare the test equipment and logbook. Complete the following steps prior to test initiation.

   a. Determine the number of test dishes needed for the test (4 replicates per site).

   b. Label each crystallizing dish with the test ID number, the site ID, the concentration (if any), and replicate (REP) number (4 REPs per site). Using a 100-mL graduated cylinder, add 25 mL of distilled water to each dish and mark the 25-mL volume level on the outside of the dish. Dispose of the distilled water.

   c. Label 8-oz. specimen containers with the test ID number, the site ID, and concentration (if any). These containers will be used to collect composite test water samples for final chemistry.

   d. At least 72 hours prior to test initiation, prepare one modified 60-cc disposable syringe per test site (to be used for water changes) as follows:

      1. Using non-toxic aquarium silicone sealant as an adhesive, affix a small piece of 100-μm Nitex mesh to one end of a 1" long, ¼" ID cylinder. Allow this to cure ~24 hours.

      2. Place thse modified plastic cylinders in distilled water and let soak for ~24 hours. Allow them to air dry for ~24 hours.

      3. Affix a 2 cm length of Tygon® tubing (1/8" I.D. x 1/4" O.D. x 1/16" wall) to the syringe tip, then affix the modified plastic cylinder to the protruding piece of tubing. Wrap with Parafilm® (if necessary) to secure both pieces together.

      4. Label these modified syringes with the test ID number, the site ID, and concentration (if any).

   e. Prepare a work table with a light board, a set of 3 sieves (#20, #40 and #60), a large plastic funnel, a 20-L waste carboy, pasteur pipettes, 3 counting dishes, and 1-oz. disposable plastic beakers for each technician participating in test termination. Prepare an appropriate volume of control water along with the associated carboy, 600-mL beakers and clean syringes for use with the control water.

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

f. Glue the following logsheets into the registered *Hyalella* Sediment Toxicity Test logbook:
a "*Hyalella azteca* Whole Sediment Toxicity Test - Test Information Logsheet,"
a "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information Logsheet," and
an appropriate number of "*Hyalella azteca* Whole Sediment Toxicity Test - Test Survival Logsheets"
Initial and date each sheet such that half of the writing extends onto the logbook page.

g. Glue an appropriate number of "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Chemistry Data Logsheets" into a registered logbook appropriate for recording chemistry analysis. Initial and date each sheet such that half of the writing extends on to the logbook page.

h. Use the table in the "*Hyalella azteca* Whole Sediment Toxicity Test - Test Information Logsheet" to record minor test non-conformities and minor deviations from protocol at any time throughout the test.

2. Prepare the sediment. Perform this procedure ~24 hours prior to test initiation. NOTE: THIS PROCEDURE MUST BE PERFORMED WEARING NITRILE GLOVES AND INSIDE A FUME HOOD.

a. Using a clean spatula or a clean 60-cc disposable plastic syringe, transfer subsamples of a selected, homogenized sediment sample to a crystallizing dish. Tap the dish on a soft surface to dispense the sediment evenly across the bottom of the dish. Continue to add sediment until the volume of sediment reaches the 25-mL mark on the dish.

b. Using a 100-mL graduated cylinder, add 100 mL of control water to the dish. Slowly pour the water down the inside of the dish to avoid disturbing the sediment.

c. Repeat steps 2.a and 2.b three more times per site for a total of four replicates per site or concentration.

d. Remove the four dishes from the fume hood and place them in a water bath or environmental chamber (maintained at 24 ± 2°C with a 16:8 hour light:dark photoperiod).

e. Replace the unused sediment sample and select a new homogenized sediment sample.

f. Repeat steps 2.a to 2.e for all remaining sediment site or concentration samples. When all test dishes have been prepared, cover the dishes with a plexiglass sheet to reduce evaporation. Allow the sediment to settle ~24 hours prior to adding the test organisms.

3. Collect and pre-count test organisms as per SOP-25.

4. To initiate the test, complete the following.

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

a. Record the following information on the "*Hyalella azteca* Whole Sediment Toxicity Test - Test Information Logsheet":
the test ID number,
the test initiation date (DAY 0),
the test termination date (DAY 10),
the list of sample site designations (i.e., river mile),
the site ID numbers,
a check mark for the appropriate control water type,
the batch number (if applicable),
a check mark for the appropriate test animal source, and
the age and/or size of the test organisms on the date of DAY 0, and
the culture tank number from which they were collected.

b. Record the following information on the "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information Logsheet":
the test ID number,
the test initiation date,
the test termination date,
your initials and the date under DAY 0, and
the time the first organisms are added to the prepared test dishes.

c. Record the temperature of the water bath or environmental chamber and the thermometer number under "DAY 0 Temperature Information." Measure and average the temperature of the overlying water of 4 randomly selected test dishes. Record this under "DAY 0 Temperature Information," also.

d. Remove both the prepared test dishes (with sediment) and the known-size or known-age culture tanks from the water bath or environmental chamber.

e. Fill 1-oz. disposable plastic beakers with ~5 mL of control water. Using a transfer pipet, randomly transfer 5 test animals from the culture tanks to the beaker. Each test chamber will receive 20 test animals; therefore, 4 beakers containing 5 test animals will be needed for each test chamber. Select only test animals that appear active and healthy.

f. Transfer 20 animals to a randomly selected test chamber using a transfer pipet. Add the animals to the test chambers in one at a time. Introduce them below the water surface to minimize air entrapment and avoid producing "floaters". This provides for two counts. Keep the amount of water transferred to a minimum.

g. Repeat steps 2.e and 2.f for the remaining test chambers.

h. Add 1 mL of *Hyalella* food to each test chamber.

i. Record the time the last animal was added on the "*Hyalella azteca* Whole Sediment Toxicity Test -Daily Test Information logsheet." Record the feeding time, *Hyalella* food date and

volume fed and the control water batch number on the "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information logsheet."

j. Return the test chambers to the water bath or environmental chamber and cover with a plexiglass sheet to reduce evaporation.

k. Inspect the test chambers approximately 1-2 hours after the *Hyalella* are added to ensure that no animals are trapped in the surface tension of the overlying water. Discard these "floaters" and replace with new test animals from the culture tanks.

5. Perform a 60% renewal of the overlying water on Days 1, 3, 5, 7, and 9.

a. Record your initials and the date under the appropriate test day. Record the bath or chamber temperature, test chamber temperature, and thermometer number and the start time on the "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information Logsheet."

b. Remove the test chambers from the water bath or environmental chamber.

c. Using the modified 60-cc syringe, carefully draw off slightly more than 60 mL (e.g., 62 mL) of overlying water from a selected test chamber. Carefully dispense the excess test water volume back into the test chamber such that the final volume of test water remaining in the 60-cc syringe is 60 mL. This is to wash any animals that might cling to the mesh back into the test chamber.

d. Collect the water in the labeled 8-oz specimen container. Use the same 8-oz. container for all 4 REPs of each site/treatment. This water will be used to determine final chemistry.

e. Repeat steps 5.c and 5.d for the remaining 3 REPs.

f. Using a clean 60-cc syringe or a 100 mL graduated cylinder, slowly add 60 mL of control water down the inside of a test container. Try to avoid disturbing the sediment unnecessarily. Repeat this procedure for the remaining 3 REPs.

g. Repeat steps 5.c through 5.f for the remaining test sites/concentrations.

h. Add 1 mL of *Hyalella* food to each test chamber. Record the water renewal ending times, *Hyalella* food date and volume fed, feeding time, and control water batch number on the "*Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information Logsheet."

i. Return the test chambers to the water bath or environmental chamber and cover with a plexiglass sheet.

6. Terminate the test on Day 10.

a. Perform steps 5.a to 5.e with one set of 4 test chambers of a selected site/concentration.

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

b. Sieve the test chambers to recover the test organisms.

(1) Add ~10 mL of distilled water to each of several (e.g., 4) 1-oz disposable plastic beakers.

(2) Select one test chamber.

(3) Gently swirl the water in the chamber so as to resuspend the top 1-2 cm layer of sediment in the chamber.

(4) Pour this slurry through a sieve stack (a #20 ASTM sieve on top of a #40 ASTM sieve on top of a #60 ASTM sieve) secured over a funnel and plastic carboy. Temporarily set the test chamber aside.

(5) Rinse all loose sediment through the sieves with distilled water.

(6) Carefully rinse the material retained by each of the 3 sieves with distilled water into 3 separate crystallizing dishes (called "counting dishes"). Thoroughly and carefully inspect each sieve for any clinging test organisms and rinse them into the respective dish.

(7) Select one counting dish.

(8) Place the counting dish on a light board and thoroughly inspect its contents for live or dead *Hyalella*. Pipet any live organisms found into one of the disposable plastic beakers. To simplify the counting, do not add more than 5 organisms per plastic beaker. Gentle prodding of the dish's contents and several minutes of observation may be required to recover all organisms. NOTE: *Hyalella* will cling and may be attached to, or hiding beneath, sediment particles and other debris.

(9) Pipet dead organisms into a separate disposable plastic beaker. Very gently prod the organisms to ensure that it is indeed dead and not just inactive. A dissecting microscope can be used to distinguish between dead and live organisms. NOTE: Do not confuse discarded exoskeletons with dead *Hyalella*.

(10) Repeat steps 6.b.(7) to 6.b.(9) with the two remaining counting dishes.

(11) Note the total number of organisms recovered.

(a) If the recovery is less than 100% (i.e., fewer than the initial number of organisms added to the test chamber are actually found), the missing organisms are assumed to be either on one of the sieves or still in the replicate test chamber containing the rest of the sediment sample, and both must be rechecked.

(b) If all organisms are recovered, skip steps 6.b.(12) and 6.b.(13) and proceed to step 6.c.

(12) If necessary, carefully and thoroughly recheck each sieve individually for any clinging organisms. Submerging the sieve in a 4-L plastic tank containing a layer of distilled water may encourage any clinging organism to swim free.

(13) If necessary, add ~20 mL of distilled water to the sediment in the test chamber and repeat steps 6.b.(3) to 6.b.(11) until either:

(a) all missing organisms are accounted for (i.e., all organisms are recovered), or,

(b) the entire sediment sample in that replicate test chamber has been thoroughly sieved and examined, and the missing organisms are not recovered.

c. Record the final totals of live, dead and missing organisms for that replicate test chamber in the appropriate column on the "*Hyalella azteca* Whole Sediment Toxicity Test - Test Survival Logsheet."

d. Discard the contents of the 3 counting dishes according to approved procedures. Reassemble the sieve stack and prepare the work area for the next replicate.

e. Repeat steps 6.b to 6.d for the 3 remaining replicate test chambers for that site/concentration.

f. Repeat steps 6.a to 6.e for the remaining site/concentrations.

g. After survival has been counted, confirmed and recorded for all the test chambers, discard the test organisms and sediment according to approved procedures.

7. Test acceptability is based on 80% or greater survival in the controls acceptable organism response in the Reference Toxicity Test, or ASTM E-1383-90 guidelines. If the test does not meet the criteria, notify the Project Leader for guidance.

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

8. Make a Xerox® copy of the applicable test logsheets. Place the copy in the backup *Hyalella* test notebook.

Approval

All Standard Operating Procedures generated by, revised by, and/or applicable to the ESD Toxicology Laboratory must receive the signed approval of the Group Leader.

Approved by Lynn A. Kszos (Group Leader) 1/14/94 (Date)

Effective Date 2/15/94

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

Hyalella azteca Whole Sediment Toxicity Test
Test Information Logsheet

Test Identification Number: _____ Test Initiation Date: _____

Technician(s) Initials: _____ Test Termination Date: _____

Site ID	ID Nos.
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____
_____	_____

Control Water Type (✓)

30% Dilute Mineral Water + Trace Metals ☐

DMW Batch Numbers used: _____

Test Animal Information

Source of *Hyalella azteca* (✓):

ESD Aquatic Toxicology Laboratory Cultures ☐

Culture Tank Number(s) used: _____

Size/Age of *Hyalella azteca* on _____ (date):

Size Range: _____ to _____ µm

OR

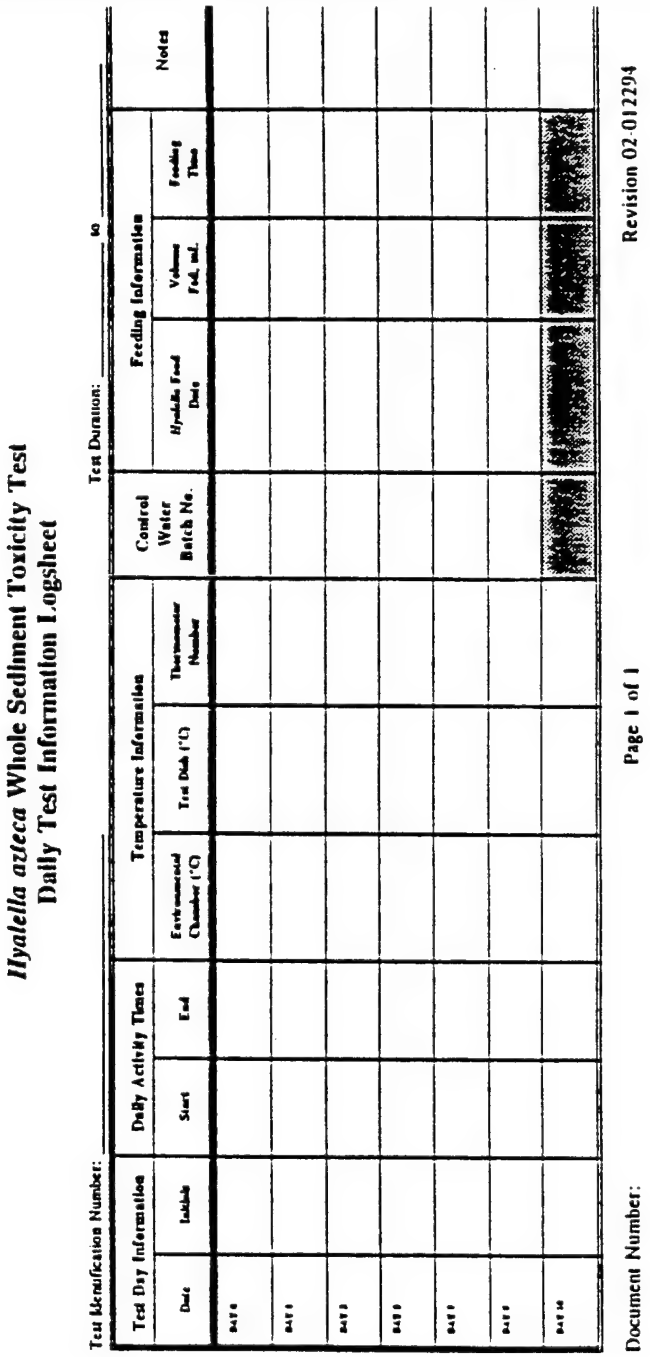
Age: _____ to _____ days old

Organisms per REP: _____ ; REPs per treatment: _____

Record of Minor Test Non-Conformances		
Date	Description of Non-Conformity	Initials

Exhibit SOP-21.1. *Hyalella azteca* Whole Sediment Toxicity Test - Test Information Sheet

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST



***Hyalella azteca* Whole Sediment Toxicity Test**
Daily Test Information Logsheet

Test Identification Number: _____ Test Duration: _____ to _____

Test Day Information		Daily Activity Times		Temperature Information			Control Water Batch No.	Feeding Information			Notes
Date	Initials	Start	End	Environmental Chamber (°C)	Test Dish (°C)	Thermometer Number		Hyalella Food Date	Volume Fed, mL	Feeding Time	
Date 0											
Date 1											
Date 2											
Date 3											
Date 4											
Date 5											
Date 10											

Page 1 of 1

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Exhibit SOP-21.2. *Hyalella azteca* Whole Sediment Toxicity Test - Daily Test Information Logsheet

Hyalella azteca Whole Sediment Toxicity Test
Test Survival Logsheet

Test Number: ______________ Test Dates: __________ to __________

			REP 1	REP 2	REP 3	REP 4	Totals
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____
Stn/Conc.	Sample ID Number	# Alive					Alive:
		# Dead					____
		# Missing					Recovery:
		Recovery					____

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Exhibit SOP-21.3. *Hyalella azteca* Whole Sediment Toxicity Test - Test Survival Logsheet

ESD TOXICOLOGY LABORATORY
QUALITY ASSURANCE MANUAL

SECTION SOP-21
PAGE 12 of 12
DATE 02-01-94

SUBJECT: *HYALELLA AZTECA* WHOLE SEDIMENT TOXICITY TEST

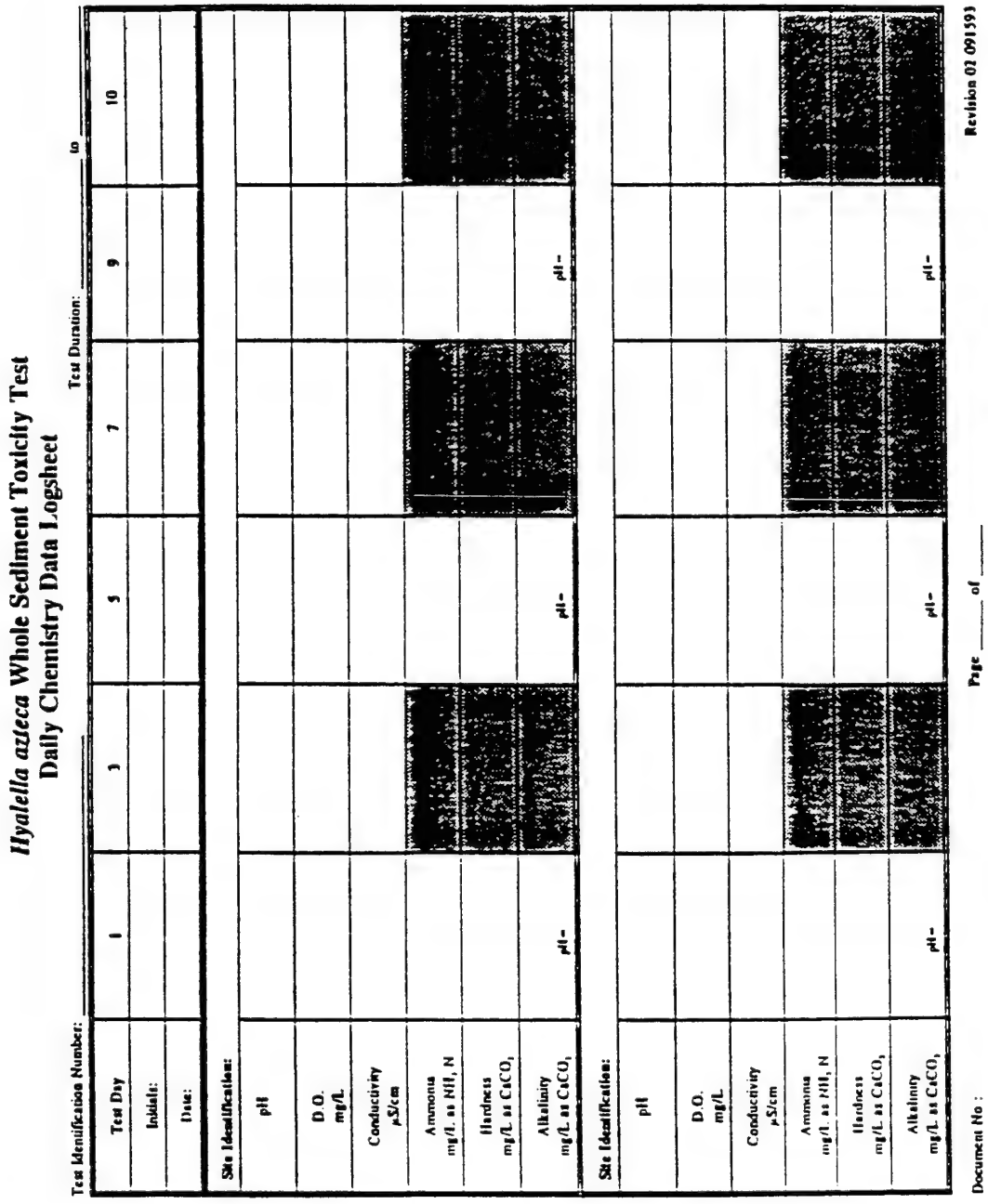

Exhibit SOP-21.4. *Hyalella azteca* Whole Sediment Toxicity Test - Daily Chemistry Data Logsheet

Draft Standard Operating Procedure

- **Subject:** *Elimia clavaformis* 72-h static renewal feeding test

Purpose: To estimate toxicity of water-borne contaminants by their action on the feeding rate of the freshwater snail, *Elimia clavaeformis*.

Equipment:

Fresh green leaf lettuce
#14 cork hole puncher
Standard office stapler/staples
8" X 10" piece of cardboard
Forceps
1 liter glass jar(s)
500 mL acid-washed beakers (3 per treatment or site)
Nylon mesh
Rubber bands
Paper wipes
White plastic tray (11" X 14")
Plastic-lined laboratory paper
Mettler balance (e.g., AE 163)
Spring/well water or 25% DMW (reference water)
Water bath or other temperature controlled system (e.g. environmental chamber)
5 gallon aquarium(s)
Photolight
1-oz. disposable plastic beakers
Permanent ink marker (e.g., Sharpie)
Thermometer (± 0.5° C)
2-gal. plastic bucket
Ceramic tiles or rocks
Deionized distilled water
Weighing Paper
1000mL Erlenmeyer flask
Lotus 1-2-3
SAS (or analogous statistical analysis software)
E. clavaeformis—Daily Test Information Logsheet
72-h static-renewal feeding test—Equipment Data sheet

Definitions:

Treatment - any substance or condition which might be controlled or manipulated and thus modifying the food consumption rate of Elimia clavaformis. Examples of treatments could include site (from which water is obtained), chemicals, pH, temperature, etc.

Control - the type of control water to be used depends on the purpose of the experiment. If stream

waters are being tested, then spring/well water or 25% dilute mineral water (DMW) may be used as the control. If several dilutions of a chemical are being tested, 25% DMW should be used for the control and to prepare test solutions.

Procedure:

1. Collect *Elimia clavaeformis* (medium size, about 300-350 mg blotted wet weight) from an appropriate reference site, (e.g., in the vicinity of the weir downstream from WCK 6.8). Place the snails in a bucket containing the reference stream water. Measure the temperature of the water, record the temperature on the Daily Test Information Logsheet, and take the snails to the laboratory.

2. In the laboratory, set the temperature of a water bath to the temperature of the reference site. Place the bucket with the snails into the water bath. Also, place a small glass aquarium into the water bath and fill 3/4 full with control water. Allow the water to equilibrate to the set temperature.

3. Record on the Daily Test Information Logsheet the type of treatment and the type of control water used in the experiment.

4. Pre-rinse the fresh green leaf lettuce with distilled water. Place the snails from the bucket into the aquarium and add one or two leaves of the lettuce. Use ceramic tiles or one or two rocks to hold the lettuce on the bottom, thus making it accessible to the snails.

5. Acclimate the snails to 25 ± 2 °C by adjusting the temperature of the water 2°C per day; adjust the temperature 1°C in the morning and 1°C in the evening.

6. Each morning during the acclimation period, prepare another small aquarium containing fresh control water and allow it's to equilibrate to the present water temperature. Then transfer the snails from the aquarium with the old water into the aquarium containing fresh control water. Add fresh lettuce as described above.

7. Before starting a feeding test, paste a Daily Test Information Sheet for each beaker into a registered logbook. (An example of the Daily Test Information Logsheet and what to record on it is provided in this SOP).

8. Record identification information from water baths, balances, thermometers, and any other electronic equipment to be used on the Static-Renewal Feeding Test—Equipment Data sheet. Record who calibrated the instruments, and the frequency or instrument calibration.

9. Label a medium jar with "lettuce soaking in water", the date and your initials. Add about 500 mL of control water used for the test to this jar.

10. On the morning when the test is to be started, cut out three lettuce discs per beaker (plus several extra). For this, use a #14 cork hole puncher and a piece of cardboard to protect the counter top surface. Cut the discs from near the outer edge of the leaf to create homogeneous discs. Hold lettuce disc with a pair of forceps, and staple it twice, distributing the staples evenly. The staples add enough weight so that the disks will not float. Repeat this step for all discs. Place the stapled lettuce discs into the jar with control water, label the jar, and place the jar in a

refrigerator for 2-3 hours to allow the discs to hydrate.

11. Line a large plastic tray with plastic-lined lab paper. Mark the paper in a grid using a permanent-ink marker to keep track of lettuce discs for each treatment (see Fig. 1).

Fig.1

	Treatment 1	Treatment 2	Treatment 3
Replicate 1			
Replicate 2			
Replicate 3			

12. Obtain one 500-mL beaker for each replicate; rinse the beaker with deionized distilled water. Label each beaker with the type of treatment and replicate number using a permanent ink marker. Color labels can be used as an aid in identifying the various treatments. Add 250 mL of the appropriate control water to each beaker and place the beakers in a 25±2°C water bath with an overhead photolight (16:8 light:dark).

13. About 1 h before starting the test, moisten the plastic-lined paper in the plastic tray with control water to prevent desiccation of the lettuce discs. Remove the lettuce discs from the refrigerator. Fold several paper towels in half and place three (on top of each other) next to the balance.

14. Before weighing the lettuce discs, first check the balance level; then check balance zero; then verify the accuracy of the balance by use of a 200 or 500-mg standard weight. Record the weight on the Daily Test Information Logsheet. Place a piece of weighing paper on the balance and zero.

15. Remove three lettuce discs, with forceps, from the jar and place them upon the paper towels. In a consistent manner, blot the lettuce discs 3 to 4 times with paper towels to remove excess water. Using forceps, place the three lettuce discs (and their attendant staples) on the balance and record the weight on the Daily Test Information Logsheet for a specific treatment and replicate. Remove the lettuce discs from the balance and place them into the plastic tray on the corresponding square for that treatment and replicate. Replace the weighing paper and re-zero the balance after each measurement.

16. Check the water temperature in the test beakers. If the temperature has equilibrated with the water bath temperature, measure and record the temperature for the water bath and each beaker on the Daily Test Information Logsheet. Adjust the waterbath temperature if needed.

17. Place 12 snails in each beaker, then add the appropriate set of three weighed lettuce discs.

Place a piece of mesh over the top of the beaker and secure the mesh using a rubber band. Place the beaker back into the water bath.

18. The following day, prepare new lettuce discs as described in steps 11-14. Also, prepare fresh treatment water by pouring water into clean labeled 1000-mL containers (e.g., Erlenmeyer flasks) and placing them into the water bath.

19. 24, 48, and 72 h after starting the test, record the temperature of the water bath on the Daily Test Information Logsheet.

20. For each replicate, remove uneaten lettuce (**and staples!**) and place these items in the appropriate square on the plastic tray. Be sure to recover even small pieces of lettuce that may have become detached from the original discs. Holding the mesh over the beaker, gently swirl the water remaining in the beaker to suspend particulate matter, and pour out the old water from each beaker. Add fresh treatment water and fresh stapled lettuce discs. Place the mesh over the beaker and return it to the water bath.

21. Weigh each group of three old lettuce discs (and then staples) using the procedure described in step 14. Record the weights on the appropriate Daily Test Information Logsheets.

22. Repeat steps 17-20 at 48-h and repeat steps 18-20 at 72-h.

23. Enter weight data into a computer spreadsheet (i.e., Lotus 1-2-3) and calculate the amount of lettuce eaten for each day (i.e., the difference between the lettuce weight before and after 24-h).

24. Differences in the feeding rates among the treatments can be analyzed using SAS-GLM with a repeated-measure subroutine (SAS 1985 a,b).

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Nellis AFB, NV 89191-6546
ATTN: WTC/EVR
Offutt AFB, NE 68113-4019
ATTN: 55 CES/CEV
Pope AFB, NC 28308-2890
ATTN: 23 CES/CEV
Mountain Home AFB, ID 83648-5442
ATTN: 366 CES/CEV
Seymour Johnson AFB, NC 27531-2355
ATTN: 4 CES/CEV
Shaw AFB, SC 29152-5123
ATTN: 20 CES/CEV
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